Europäisches Patentamt | European Patent Office | Office européen des brevets

Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

03290812.1 ✓

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

Anmeldung Nr:
Application no.: 03290812.1
Demande no:

Anmeldetag:
Date of filing: 31.03.03
Date de dépôt:

Anmelder/Applicant(s)/Demandeur(s):

CropDesign N.V.
Technologiepark 3
9052 Zwijnaarde-Gent
BELGIQUE
CENTRE NATIONAL DE
LA RECHERCHE SCIENTIFIQUE (CNRS)
3, rue Michel Ange
75016 Paris
FRANCE

Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

Plants having modified growth characteristics and a method for making the same

In Anspruch genommene Priorität(en) / Priority(ies) claimed /Priorité(s) revendiquée(s)
Staat/Tag/Aktenzeichen/State/Date/File no./Pays/Date/Numéro de dépôt:

Internationale Patentklassifikation/International Patent Classification/
Classification internationale des brevets:

C12N15/00

Am Anmeldetag benannte Vertragstaaten/Contracting states designated at date of filing/Etats contractants désignées lors du dépôt:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL
PT RO SE SI SK TR LI

083-CCS52-PROV

Plants having modified growth characteristics and a method for making the same

The present invention concerns a method for modifying plant growth characteristics. More specifically, the present invention concerns a method for modifying plant growth characteristics by modulating, in a plant, expression of a cell cycle switch gene encoding a 52kDa protein (CCS52 protein) and/or activity of the CCS52 protein itself. The present invention also concerns plants having modulated expression of a nucleic acid sequence encoding a CCS52 protein and/or modulated activity of a CCS52 protein, which plants have modified growth characteristics relative to corresponding wild type plants.

Given the ever-increasing world population, and the dwindling supply of arable land available for agriculture, it remains a major goal of agricultural research to improve the efficiency of agriculture. Conventional means for crop and horticultural improvements utilise selective breeding techniques to identify plants having desirable characteristics. However, such selective breeding techniques have several drawbacks, namely that these techniques are typically labour intensive and result in plants that often contain heterogenous genetic complements that may not always result in the desirable trait being passed on from parent plants. Advances in molecular biology have allowed mankind to manipulate the germplasm of animals and plants. Genetic engineering of plants entails the isolation and manipulation of genetic material (typically in the form of DNA or RNA) and the subsequent introduction of that genetic material into a plant. Such technology has led to the development of plants having various improved economic, agronomic or horticultural traits. A trait of particular economic interest is high yield.

It has now been found that plant growth characteristics may be modified by increased expression in a plant of a nucleic acid encoding a CCS52 protein.

CCS52 belongs to a small group of proteins containing several WD repeat motifs (see published International patent application WO 99/64451). CCS52 is a plant homologue of animal APC activators involved in mitotic cyclin degradation. Cebolla *et al.* (EMBO J 1999 Aug. 16;18: 4476-84) reported the isolation of CCS52 clones from *Medicago sativa* root nodules. They also report CCS52 to be a small multigenic family that appears to be conserved in plants. In yeast, overexpression of CCS52 was shown to lead to mitotic cyclin degradation, cell division arrest, endoreduplication and cell enlargement. In *Medicago*, CCS52 was found in differentiating cells undergoing endoreduplication. Overexpression of the gene in the antisense

orientation resulted in partial suppression of CCS52 expression, decreased number of endocycles and decreased volume of the largest cells. In WO 99/64451, *Medicago* plants expressing an antisense version of the CCS52 gene from *Medicago* were reported to be slender with less lateral branches, bigger cells in petioles and reduced endoploidy in petioles, hypocotyls and roots. In this document it is suggested that downregulation of CCS52 expression pushes the cells towards proliferation and that overproduction of CCS52 pushes the cells towards differentiation. Although it was suggested that expression in plants of the medicago CCS52 gene under control of the Cauliflower mosaic virus 35S promoter should result in a positive effect on somatic embryogenesis (WO99/64451), such evidence has not been provided so far (E. Kondorosi personal communication). To the contrary, there is now provided evidence that overexpression of the same medicago gene CCS52 under control of the 35S promoter was found to be lethal in *Medicago* (E. Kondorosi, personal communication). It has been seen that CCS52 under the control of the 35S promoter interferes with somatic embryogenesis of transgenic plants and has therefore lethal effects on the transgenic plant. Additionally, there is now provided evidence that in *Arabidopsis* the Arabidopsis CCS52 gene leads to small and aberrant plants when expressed under control of the 35S promoter (see example 6).

Also in the document Kondorosi E. et al. (1999, The EMBO Journal vol.18 no.16, p.4476-4484) it is stated that the CCS52 product may switch proliferating cells to differentiation programmes, and for some cells differentiation means to undergo endocycles. This switch to differentiation (or endoreduplication) clearly involves an arrest in proliferation, thus an arrest in cell division. These data were in line with earlier findings in yeast and teach that when CCS52 is used to increase differentiation such as endoreduplication, a cell cycle arrest is inevitably triggered. Thus the beneficial effect of endoreduplication on the one hand, namely the increased cell size, would be levelled out by the reduction of cell number due to the cell division arrest. The results obtained in *Medicago* and *Arabidopsis*, with CCS52 overexpression driven by the 35S promoter corroborated this view.

Surprisingly it has now been demonstrated in plants that the use of CCS52 in addition to increased endoreduplication, also leads to enhanced cell division, the combination of both effects resulting in improved plant growth characteristics.

It has now been found that plant growth characteristics such as plant size and organ size may be modified by increased expression in a plant of a nucleic acid encoding a CCS52 protein. It has now been found how the CCS52 gene can be used successfully to stimulate cell division and cell division rate and thus to increase cell numbers. Additionally it has been demonstrated

that these processes can be positively influenced in combination with enhanced endoreduplication. Advantageously, in the methods of the present invention it has been shown how to combine endoreduplication with enhanced cell division, instead of causing cell division arrest as is normally expected with endoreduplication.

According to a first embodiment of the present invention, there is provided a method for modifying the growth characteristics of a plant, comprising increased expression in a plant of a nucleic acid sequence encoding a CCS52 protein and/or modulating activity in a plant of a CCS52 protein.

Modulated (increased) expression of a nucleic acid sequence encoding a CCS52 protein or modulation of the activity of the CCS52 protein itself encompasses altered expression of a gene and/or altered levels of a gene product, namely a polypeptide, in specific cells or tissues.

Advantageously, modulation (increase) of expression of a nucleic acid sequence encoding a CCS52 protein and/or modulation of activity of the CCS52 protein itself may be effected by chemical means, i.e. by exogenous application of one or more compounds or elements capable of modulating activity of the CCS52 protein and/or capable of modulating expression of a CCS52 gene (which may be either an endogenous gene or a transgene introduced into a plant). The exogenous application may comprise contacting or administering cells, tissues, organs or organisms with the gene product or a homologue, derivative or active fragment thereof and/or to antibodies to the gene product. Such antibodies may comprise "plantibodies", single chain antibodies, IgG antibodies and heavy chain camel antibodies, as well as fragments thereof. Modulation of expression of a nucleic acid sequence encoding a CCS52 protein and/or modulation of activity of the CCS52 protein itself may also be effected as a result of decreased levels of factors that directly or indirectly activate or inactivate a CCS52 protein. Additionally or alternatively, contacting or administering cells, tissues, organs or organisms with an interacting protein or to an inhibitor or activator of the gene product provides another exogenous means for modulation of expression of a nucleic acid sequence encoding a CCS52 protein and/or for modulation of activity of the CCS52 protein itself.

Therefore, according to one aspect of the present invention, there is provided a method for modifying the growth characteristics of a plant, comprising exogenous application of one or more compounds or elements capable of modulating (increase) expression of a CCS52 gene and/or capable of modulating activity of a CCS52 protein.

Additionally or alternatively, and according to a preferred embodiment of the present invention, modulation (increase) of expression of a nucleic acid sequence encoding a CCS52 protein and/or modulation of activity of the CCS52 protein itself may be effected by recombinant means. Such recombinant means may comprise a direct and/or an indirect approach for modulation of expression of a nucleic acid sequence and/or for modulation of the activity of a protein, a direct approach being, for example, when a plant is transformed with a gene encoding a CCS52 protein and an indirect approach being, for example, when a plant is transformed with a gene which is able to have an effect on a CCS52 protein already in that plant (which CCS52 protein may be an endogenous gene or a gene (previously) introduced into the plant).

For example, an indirect approach may comprise introducing, into a plant, a nucleic acid sequence capable of modulating activity of the protein in question (a CCS52 protein) and/or expression of the gene in question (a gene encoding a CCS52 protein). The CCS52 gene or the CCS52 protein may be wild type, i.e. the native or endogenous nucleic acid or polypeptide. Alternatively, it may be a nucleic acid derived from the same or another species, which gene is introduced as a transgene, for example by transformation. This transgene may be substantially modified from its native form in composition and/or genomic environment through deliberate human manipulation. Also encompassed by an indirect approach for modulating activity of a CCS52 protein and/or expression of a CCS52 gene is the inhibition or stimulation of regulatory sequences that drive expression of the native gene or transgene. Such regulatory sequences may be introduced into a plant.

A direct and preferred approach on the other hand comprises introducing into a plant a nucleic acid sequence encoding a CCS52 protein or a homologue, derivative or active fragment thereof. The nucleic acid sequence may be introduced into a plant by, for example, transformation. The nucleic acid sequence may be derived (either directly or indirectly (if subsequently modified)) from any source provided that the sequence, when expressed in a plant, leads to modulated activity of a CCS52 protein. The nucleic acid sequence may be isolated from a microbial source, such as bacteria, yeast or fungi, or from a plant, algal or animal (including human) source. This nucleic acid may be substantially modified from its native form in composition and/or genomic environment through deliberate human manipulation.

Therefore according to a preferred aspect of the present invention, there is provided a method for modifying the growth characteristics of a plant, comprising introducing into a plant cell, tissue or organ, an isolated nucleic acid sequence capable of modulating CCS52 gene

expression levels and/or CCS52 protein activity, preferably wherein the isolated nucleic acid sequence encodes a CCS52 protein.

The isolated nucleic acid sequence is preferably a homologous nucleic acid sequence, i.e. a nucleic acid sequence obtained from a plant, whether from the same plant species or different. Further preferably, the nucleic acid sequence is isolated from a dicotyledonous plant, preferably *Arabidopsis*. More preferably, the nucleic acid is as represented by SEQ ID NO: 1 (AtCCS52A1 gene) or a functional portion thereof, or a nucleic acid sequence capable of hybridising therewith, or is a nucleic acid sequence encoding an amino acid sequence represented by SEQ ID NO: 2 (AtCCS52A1 protein) or a homologue, derivative or active fragment thereof.

The term CCS52 nucleic acid sequence/gene, as defined herein, refers to a nucleic acid sequence as represented by SEQ ID NO: 1 or a portion thereof or to nucleic acid sequences capable of hybridising therewith, which hybridising sequences encode proteins having CCS52 activity, i.e. similar biological activity to that of SEQ ID NO: 1, and to nucleic acid sequences encoding an amino acid sequence represented by SEQ ID NO: 2 or a homologue, derivative or active fragment thereof.

Advantageously, the method according to the present invention may also be practised using portions of a sequence represented by SEQ ID NO: 1 or by using sequences that hybridise (preferably under stringent conditions) to SEQ ID NO: 1 (which hybridising sequences encode proteins having CCS52 activity), or by using homologues, derivatives or active fragments of a sequence according to SEQ ID NO: 2. Suitable homologues of SEQ ID NO: 2 include a rice homologue represented by SEQ ID NO: 4 and 6 encoded by the nucleic acid sequence represented by SEQ ID NO: 3 and 5. The method according to the invention may also be practised using sequences that hybridise (preferably under stringent conditions) to SEQ ID NO: 3 and/or 5 (which hybridising sequences encode proteins having CCS52 activity) or using portions of a sequence represented by SEQ ID NO: 3 and/or 5.

Methods for the search and identification of CCS52 protein homologues would be well within the realm of a person skilled in the art. Methods for the alignment of sequences for comparison are well known in the art, such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch (J. Mol. Biol. 48: 443-453, 1970) to find the alignment of two complete sequences that maximises the number of matches and minimises the number of gaps. The BLAST algorithm calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The software for

performing BLAST analysis is publicly available through the National Centre for Biotechnology Information. The rice homologue represented by SEQ ID NO: 4 and 6 was identified using blast default parameters and using SEQ ID NO 1 as probe sequence to search the database.

Homologues of the AtCCS52 gene/protein according to SEQ ID NO 1/2, suitable for use in the methods of the present invention, are identified from publicly available databases (such as Genbank). Examples of such homologues are presented in the phylogenetic tree in figure 12. The homologues are presented by their Genbank accession number. More preferred homologues to be usd in the present invention are the homologues that group close to SEQ ID NO 2 (AtCCS52A1_At4g22910) protein, for example those homologues that group between the OsAP003298.3 protein and the AtCCS52A1_At4g22910 protein. These homologues include but are not limited to for example Le_AW0030735, AtCCS52A2_At4g11920, MtCCS52A_AF134835, Gm_BG044933, Os_AAN74839, Zm_AY112458, AtCCS52B_At5g13840, MsCCSB, Gm_AI736659 and Zm_AI861254.

"Homologues" of a CCS52 protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or insertions relative to the unmodified protein in question and having similar biological and functional activity as the unmodified protein from which they are derived. To produce such homologues, amino acids of the protein may be replaced by other amino acids having similar properties (such as similar hydrophobicity, hydrophilicity, antigenicity, propensity to form or break α-helical structures or β-sheet structures). Conservative substitution tables are well known in the art (see for example Creighton (1984) Proteins. W.H. Freeman and Company). The homologues useful in the method according to the invention have at least 50% sequence identity or similarity (functional identity) to the unmodified protein, alternatively at least 60% sequence identity or similarity to an unmodified protein, alternatively at least 70% sequence identity or similarity to an unmodified protein. Typically, the homologues have at least 80% sequence identity or similarity to an unmodified protein, preferably at least 85% sequence identity or similarity, further preferably at least 90% sequence identity or similarity to an unmodified protein, most preferably at least 95% sequence identity or similarity to an unmodified protein.

Two special forms of homology, orthologous and paralogous, are evolutionary concepts used to describe ancestral relationships of genes. The term "parologous" relates to gene-duplications within the genome of a species leading to paralogous genes. The term "orthologous" relates to homologous genes in different organisms due to ancestral relationship. The term "homologues" as used herein also encompasses paralogues and orthologues of the proteins useful in the methods according to the invention.

"Substitutional variants" of a protein are those in which at least one residue in an amino acid sequence has been removed and a different residue inserted in its place. Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1-10 amino acid residues, and deletions will range from about 1-20 residues. Preferably, amino acid substitutions comprise conservative amino acid substitutions.

The exact function of CCS52 gene and related genes may be discovered by the use of reverse genetics, such a TILLING (Targeted Induced Local Lesions IN Genomes), by the discovery of sites and motifs crucial for the gene and protein function (McCAllum et al., 2000 Plant Physiol 2000 Jun;123(2):439-42 *Targeting induced local lesions IN genomes (TILLING) for plant functional genomics.*; Perry et al., 2003 Plant Physiol 2003 Mar;131(3):866-71*A TILLING Reverse Genetics Tool and a Web-Accessible Collection of Mutants of the Legume Lotus japonicus.*). Plants having mutant or dominant negative, or dominant positive phenotypes will be analysed and compared to identify the most interesting mutations. Comparison of phenotypes will be compared with phenotypes identified for example in QTLs (Quantitative Trait Loci) analysis, sequence information will be compared with the gene mapping included in a QTL. Both methods will be useful when combined in identifying new phenotypes of interest for crop breeding.

"Insertional variants" of a protein are those in which one or more amino acid residues are introduced into a predetermined site in a protein. Insertions can comprise amino-terminal and/or carboxy-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino- or carboxy-terminal fusions, of the order of about 1 to 10 residues. Examples of amino- or carboxy-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, $(histidine)_6$-tag, glutathione S-transferase-tag, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100 epitope, c-myc epitope, FLAG®-epitope, lacZ, CMP (calmodulin-binding peptide), HA epitope, protein C epitope and VSV epitope.

"Deletion variants" of a protein are characterised by the removal of one or more amino acids from the protein. Amino acid variants of a protein may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. Methods for the manipulation of DNA sequences to produce substitution, insertion or deletion variants of a protein are well known in the art. For example,

techniques for making substitution mutations at predetermined sites in DNA are well known to those skilled in the art and include M13 mutagenesis, T7-Gen *in vitro* mutagenesis (USB, Cleveland, OH), QuickChange Site Directed mutagenesis (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

The term "derivatives" refers to peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise substitutions, deletions or additions of naturally and non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the protein, for example, as presented in SEQ ID NO: 2. "Derivatives" of a CCS52 protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise naturally occurring altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may also comprise one or more non-amino acid substituents compared to the amino acid sequence from which it is derived, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence such as, for example, a reporter molecule which is bound to facilitate its detection, and non-naturally occurring amino acid residues relative to the amino acid sequence of a naturally-occurring protein.

"Active fragments" of a CCS52 protein encompasses at least five contiguous amino acid residues of a protein, which residues retain similar biological and/or functional activity to the naturally occurring protein.

Advantageously, the method according to the present invention may also be practised using portions of a DNA or nucleic acid sequence, which portions retain CCS52 activity, i.e. a similar biological function to that of SEQ ID NO: 2. Portions of a DNA sequence refer to a piece of DNA derived or prepared from an original (larger) DNA molecule, which DNA portion, when expressed in a plant, gives rise to plants having modified growth characteristics. The portion may comprise many genes, with or without additional control elements, or may contain just spacer sequences etc.

Suitable fragments of the proteins or suitable portions of nucleic acids that correspond to such fragments of proteins, for use in the methods of the present invention, are the portions that correspond to conserved plant proteins domains or to conserved plant-specific motifs of the CCS52 protein. Nine such domains are presented in figure 13. Preferred CCS52 homologues to be used in the present invention are plant CCS52 proteins that comprise at least 4 of these consensus motifs. Motif number nine is presumably involved in the interaction with other

proteins and is preferably present in the homologues suitable for use in the methods of the present invention. Deviation of the consensus sequence of these motifs is possible as illustrated by four different plant CCS52 proteins in figure 13. In a particular embodiment of the invention, the CCS52 protein comprises a sequence that is 43% or 53% or more identical to a consensus motif sequence as presented in figure 13. In a preferred embodiment of the present invention the CCS52 gene or protein used to improve plant growth characteristics is a CCS52A1 protein. A CCS52A1 protein being AtCCS52A1 or a protein closer related to AtCCS52A1 , than to AtCCS52A2 or AtCCS52B. Further, a preferred CCS52 protein has at least 4 of the 9 conserved motifs, that are identical to the motifs of the AtCCS52 A1 protein.

Alternatively, motifs can be used to search databases and to identify homologues sequences. Accordingly, another aspect of the present invention is to use the conserved CCS52 motifs as presented in figure 13, to identify homologues of the At CCS52 gene/protein and/or to identify proteins capable of altering plant growth characteristics in a similar way as the AtCCS52 gene/protein. Therefore, the present invention provides a method to identify new CCS52 proteins, based on the localisation of any of the consensus motif sequences as presented in figure 13 (SEQ ID NO 7 to 15) in an amino acid sequence.

The present invention also encompasses nucleic acid sequences capable of hybridising with a nucleic acid sequence encoding a CCS52 protein, which nucleic acid sequences may also be useful in practising the methods according to the invention. The term "hybridisation" as defined herein is a process wherein substantially homologous complementary nucleotide sequences anneal to each other. The hybridisation process can occur entirely in solution, i.e. both complementary nucleic acids are in solution. Tools in molecular biology relying on such a process include the polymerase chain reaction (PCR; and all methods based thereon), subtractive hybridisation, random primer extension, nuclease S1 mapping, primer extension, reverse transcription, cDNA synthesis, differential display of RNAs, and DNA sequence determination. The hybridisation process can also occur with one of the complementary nucleic acids immobilised to a matrix such as magnetic beads, Sepharose beads or any other resin. Tools in molecular biology relying on such a process include the isolation of poly (A+) mRNA. The hybridisation process can furthermore occur with one of the complementary nucleic acids immobilised to a solid support such as a nitro-cellulose or nylon membrane or immobilised by e.g. photolithography to, for example, a siliceous glass support (the latter known as nucleic acid arrays or microarrays or as nucleic acid chips). Tools in molecular biology relying on such a process include RNA and DNA gel blot analysis, colony hybridisation, plaque hybridisation, *in situ* hybridisation and microarray hybridisation. In order to allow

hybridisation to occur, the nucleic acid molecules are generally thermally or chemically denatured to melt a double strand into two single strands and/or to remove hairpins or other secondary structures from single stranded nucleic acids. The stringency of hybridisation is influenced by conditions such as temperature, salt concentration and hybridisation buffer composition. High stringency conditions for hybridisation include high temperature and/or low salt concentration (salts include NaCl and Na_3-citrate) and/or the inclusion of formamide in the hybridisation buffer and/or lowering the concentration of compounds such as SDS (detergent) in the hybridisation buffer and/or exclusion of compounds such as dextran sulphate or polyethylene glycol (promoting molecular crowding) from the hybridisation buffer. Conventional hybridisation conditions are described in, for example, Sambrook (2001) Molecular Cloning: a laboratory manual, 3rd Edition Cold Spring Harbor Laboratory Press, CSH, New York, but the skilled man will appreciate that numerous different hybridisation conditions can be designed in function of the known or the expected homology and/or length of the nucleic acid sequence. Specifically hybridising refers to hybridising under stringent conditions, i.e. at a temperature of 60°C followed by washes in 2XSSC, 0.1XSDS, and 1X SSC, 0.1X SDS. Sufficiently low stringency hybridisation conditions are particularly preferred for the isolation of nucleic acids homologous to the DNA sequences of the invention defined supra. Elements contributing to homology include allelism, degeneration of the genetic code and differences in preferred codon usage.

The methods according to the present invention may also be practised using an alternative splice variant of a nucleic acid sequence encoding a CCS52 protein. The term "alternative splice variant" as used herein encompasses variants of a nucleic acid sequence in which selected introns and/or exons have been excised, replaced or added. Such variants will be ones in which the biological activity of the protein remains unaffected, which can be achieved by selectively retaining functional segments of the protein. Such splice variants may be found in nature or can be manmade. Methods for making such splice variants are well known in the art. Therefore according to another aspect of the present invention, there is provided, a method for modifying the growth characteristics of plants, comprising modulating expression in a plant of an alternative splice variant of a nucleic acid sequence encoding a CCS52 protein and/or by modulating activity of a CCS52 protein encoded by the alternative splice variant. Preferably, the splice variant is a splice variant of the sequence represented by either SEQ ID NO: 1 or SEQ ID NO: 3.

It is known that AtCCS52A1 belongs to a multigene family. Therefore the methods according to the present invention may also be practised using a family member of a nucleic acid sequence encoding a CCS52 protein.

Advantageously, the methods according to the present invention may also be practised using allelic variants of a nucleic acid sequence encoding a CCS52 protein, preferably an allelic variant of a sequence represented by SEQ ID NO: 1, SEQ ID NO: 3 op SEQ ID NO 5. Allelic variants exist in nature and encompassed within the methods of the present invention is the use of these natural alleles. The use of these allelic variants in particular conventional breeding programmes, such as in marker-assisted breeding is also encompassed by the present invention; this may be in addition to their use in the methods according to the present invention. Such breeding programmes sometimes require the introduction of allelic variations in the plants by mutagenic treatment of a plant. One suitable mutagenic method is EMS mutagenesis. Identification of allelic variants then takes place by, for example, PCR. This is followed by a selection step for selection of superior allelic variants of the sequence in question and which give rise to altered growth characteristics in a plant. Selection is typically carried out by monitoring growth performance of plants containing different allelic variants of the sequence in question, for example, SEQ ID NO: 1. Monitoring growth performance can be done in a greenhouse or in the field. Further optional steps include crossing plants, in which the superior allelic variant was identified, with another plant. This could be used, for example, to make a combination of interesting phenotypic features. Allelic variants also encompass Single Nucleotide Polymorphisms (SNPs), as well as Small Insertion/Deletion Polymorphisms (INDELs). The size of INDELs is usually less than 100 bp). SNPs and INDELs form the largest set of sequence variants in naturally occurring polymorphic strains of most organisms.

According to another aspect of the present invention, advantage may be taken of the nucleotide sequence capable of modulating expression of a nucleic acid encoding a CCS52 protein in breeding programmes. The nucleic acid sequence may be on a chromosome, or a part thereof, chromosomes, comprising at least the nucleic acid sequence encoding the CCS52 protein and preferably also one or more related family members. In an example of such a breeding programme, a DNA marker is identified which may be genetically linked to a gene capable of modulating expression of a nucleic acid encoding a CCS52 protein in a plant, which gene may be a gene encoding the CCS52 protein itself or any other gene which may directly or indirectly influence expression of the gene encoding a CCS52 protein and/or activity of the CCS52 protein itself. This DNA marker may then used in breeding programs to select plants having altered growth characteristics.

The methods according to the present invention may also be practised by introducing into a plant at least a part of a (natural or artificial) chromosome (such as a Bacterial Artificial Chromosome (BAC)), which chromosome contains at least a gene/nucleic acid sequence

encoding a CCS52 protein (such as represented by SEQ ID NO: 1 or SEQ ID NO: 3 or 5), preferably together with one or more related gene family members. Therefore, according to a further aspect of the present invention, there is provided a method for modifying the growth characteristics of plants by introducing into a plant at least a part of a chromosome comprising at least a gene/nucleic acid encoding a CCS52 protein.

Further, the presence of multiple conserved motifs (SEQ ID NO 7 to 15) strongly suggests that CCS52 proteins are involved in multiple interactions and that several CCS52 target genes/proteins exist. For example, it is expected that CCS52 heterodimerizes with itself or similar proteins, for example members of the same family or members of the group of AtFzy proteins (see figure 12, group between AtFzy_At4g33260 to Dc_T14352) or with other binding partners. These conserved motifs were initially used to identify plant specific CCS52 proteins. But further the identification and the provision of these consensus motif sequences enables the person skilled in the art to set up experiments to identify interactions and to isolate these binding partners and CCS52 targets. For example such experiments can involve a two-hybrid screen and/or mutagenesis in these conserved motifs.

According to a preferred aspect of the present invention, enhanced or increased expression of a nucleic acid is envisaged. Methods for obtaining enhanced or increased expression of genes or gene products are well documented in the art and include, for example, overexpression driven by a exogenous promoter, the use of transcription enhancers or translation enhancers. Preferably, the nucleic acid to be overexpressed encodes a CCS52 protein, further preferably the nucleic acid sequence encoding the CCS52 protein is isolated from *Arabidopsis*, most preferably the nucleic acid sequence is as represented by any of SEQ ID NO: 1 or a portion thereof, or encodes an amino acid sequence as represented by SEQ ID NO: 2 or a homologue, derivative or active fragment thereof. Alternatively, the nucleic acid sequence encoding the CCS52 protein is as represented by SEQ ID NO: 3 or 5 or a portion thereof, or encodes an amino acid sequence as represented by SEQ ID NO: 4 or 6, or encodes a homologue, derivative or active fragment of any of the aforementioned SEQ ID Nos. It should be noted that the applicability of the invention is not limited to use of the nucleic acid represented by SEQ ID NO: 1 nor to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 2, but that other nucleic acid sequences encoding homologues, derivatives or active fragments of SEQ ID NO: 2, or portions of SEQ ID NO: 1, or sequences hybridising with SEQ ID NO: 1 may be used in the methods of the present invention.

According to another aspect of the present invention, decreased expression of a nucleic acid sequence is envisaged. Modulating gene expression (whether by a direct or indirect approach)

encompasses altered transcript levels of a gene. Increased gene expression encompasses increased transcript level and altered transcript levels can be sufficient to induce certain phenotypic effects, for example via the mechanism of cosuppression. Here the overall effect of overexpression of a transgene is that there is less activity in the cell of the protein encoded by a native gene having homology to the introduced transgene. Thus, the overall effect is that the native gene is decreased in expression. Other examples of decreasing activity of a protein in a cell are well documented in the art and include, for example, downregulation of expression by anti-sense techniques, RNAi techniques, the use of ribozymes etc. Advantageously, the methods according to the present invention may also be practised by downregulation of a nucleic acid sequence that influences the activity of the CCS52 gene/protein. Plants having modified growth characteristics such as increased plant size and/or increase organ size may be obtained by expressing a nucleic acid sequence encoding a CCS52 protein in sense orientation or may be obtained by expressing a nucleic acid sequence capable of influencing CCS52 gene/protein activity either in sense or antisense orientation. Techniques for downregulation are well known in the art. The terms "gene silencing" or "downregulation" of expression, as used herein, refer to lowering levels of gene expression and/or levels of active gene product and/or levels of gene product activity. Such decreases in expression may be accomplished by, for example, the addition of coding sequences or parts thereof in a sense orientation (if it is desired to achieve co-suppression). Therefore, according to one aspect of the present invention, the growth of a plant may be modified by introducing into a plant an additional copy (in full or in part) of a CCS52 gene already present in a host plant. The additional gene will silence the endogenous gene, giving rise to a phenomenon known as co-suppression.

Another method for downregulation of gene expression or gene silencing comprises use of ribozymes, for example as described in Atkins *et al.* 1994 (WO 94/00012), Lenee *et al.* 1995 (WO 95/03404), Lutziger *et al.* 2000 (WO 00/00619), Prinsen *et al.* 1997 (WO 97/3865) and Scott *et al.* 1997 (WO 97/38116).

Gene silencing may also be achieved by insertion mutagenesis (for example, T-DNA insertion or transposon insertion) or by gene silencing strategies as described by, among others, Angell and Baulcombe 1998 (WO 98/36083), Lowe *et al.* 1989 (WO 98/53083), Lederer *et al.* 1999 (WO 99/15682) or Wang *et al.* 1999 (WO 99/53050). Expression of an endogenous gene may also be reduced if the endogenous gene contains a mutation. Such a mutant gene may be isolated and introduced into the same or different plant species in order to obtain plants having modified growth characteristics.

According to a second embodiment of the present invention, genetic constructs and vectors to facilitate introduction and/or expression of the nucleotide sequences useful in the methods according to the invention are provided. Therefore, according to a second embodiment of the present invention, there is provided a gene construct comprising:

(i) a nucleic acid sequence capable of modulating expression of a nucleic acid encoding a CCS52 protein and/or activity of a CCS52 protein;

(ii) one or more control sequences capable of driving expression of the nucleic acid sequence of (i) other than a strong constitutive promoter; and optionally;

(iii) a transcription termination sequence.

In the document WO9964451 it was suggested to clone the CCS52 gene under control of the 35S promoter or the *endod12Ams* promoter or the *Srglb3* promoter in order to have a positive effect on differentiation and somatic embryogenesis. These promoters are disclaimed from the constructs of the present invention.

Constructs useful in the methods according to the present invention may be constructed using recombinant DNA technology well known to persons skilled in the art. The gene constructs may be inserted into vectors, which may be commercially available, suitable for transforming into plants and suitable for expression of the gene of interest in the transformed cells. Preferably, the construct or vector according to this embodiment of the present invention is suitable for expression in a plant cell, tissue, organ or whole plant and is suitable for introduction and maintenance in a plant cell, tissue, organ or whole plant.

The nucleic acid sequence capable of modulating expression of a nucleic acid encoding a CCS52 protein and/or activity of a CCS52 protein may be a nucleic acid sequence encoding a CCS52 protein or a homologue, derivative or active fragment thereof, such as any of the nucleic acid sequences described hereinbefore. A preferred nucleic acid sequence is the sequence represented by SEQ ID NO: 1 or a portion thereof or sequences capable of hybridising therewith or a nucleic acid sequence encoding a sequence represented by SEQ ID NO: 2 or a homologue, derivative or active fragment thereof.

Plants are transformed with a vector comprising the sequence of interest (i.e., the nucleic acid sequence capable of modulating expression of nucleic acid encoding a CCS52 protein), which sequence is operably linked to one or more control sequences (at least a promoter). The terms "regulatory element", "control sequence" and "promoter" are all used herein interchangeably and are to be taken in a broad context to refer to regulatory nucleic acid sequences capable of effecting expression of the sequences to which they are ligated. Encompassed by the

aforementioned terms are transcriptional regulatory sequences derived from a classical eukaryotic genomic gene (including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence) and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. Also included within the term is a transcriptional regulatory sequence of a classical prokaryotic gene, in which case it may include a –35 box sequence and/or –10 box transcriptional regulatory sequences. The term "regulatory element" also encompasses a synthetic fusion molecule or derivative which confers, activates or enhances expression of a nucleic acid molecule in a cell, tissue or organ. The terms "control sequence", "regulatory sequence", "regulatory element" and "promoter" are used interchangeably herein. The term "operably linked" as used herein refers to a functional linkage between the promoter sequence and the gene of interest, such that the promoter sequence is able to initiate transcription of the gene of interest.

Advantageously, any type of promoter other than a strong constitutive promoter may be used to drive expression of the nucleic acid sequence depending on the desired outcome. For example, a meristem-specific promoter, such as the rnr (ribonucleotide reductase), cdc2a promoter and the cyc07 promoter, could be used to effect expression in all growing parts of the plant, thereby increasing cell proliferation, which in turn would increase yield or biomass. If the desired outcome would be to influence seed characteristics, such as the storage capacity, seed size, seed number, biomass etc., then a seed-specific promoter, such as p2S2, pPROLAMIN, pOLEOSIN could be selected. An aleurone-specific promoter may be selected in order to increase growth at the moment of germination, thereby increasing the transport of sugars to the embryo. An inflorescence-specific promoter, such as pLEAFY, may be utilised if the desired outcome would be to modify the number of flower organs. To produce male-sterile plants one would need an anther specific promoter. To impact on flower architecture for example petal size, one could choose a petal-specific promoter. If the desired outcome would be to modify growth and/or developmental characteristics in particular organs, then the choice of the promoter would depend on the organ to be modified. For example, use of a root-specific promoter would lead to increased growth and/or increased biomass or yield of the root and/or phenotypic alteration of the root. This would be particularly important where it is the root itself that is the desired end product, such crops including sugar beet, turnip, carrot, and potato. A fruit-specific promoter may be used to modify, for example, the strength of the outer skin of the fruit or to increase the size of the fruit. A green tissue-specific promoter may be used to increase leaf size. In a preferred embodiment of the invention a cell wall-specific promoter may be used to increase the rigidity of the cell wall, thereby increasing pathogen resistance. An

anther-specific promoter may be used to produce male-sterile plants. A vascular-specific promoter may be used to increase transport from leaves to seeds. A vascular-specific promoter may be used also to (further) increase the stem size when used to drive the CCS52 gene in a plant. The latter is particularly important where it is the stem that is the desired end product, such plants including amongst others sugar cane and trees such as poplar, eucalyptus and pine. A nodule-specific promoter may be used to increase the nitrogen fixing capabilities of a plant, thereby increasing the nutrient levels in a plant. A stress-inducible promoter may also be used to drive expression of a nucleic acid to increase growth rate during conditions of stress. In a preferred embodiment a promoter specifically expressed in an epidermal outgrowth tisse, is used to drive the expression of the CCS52 gene in the methods of the present invention.

For the methods of the present invention, the nucleic acid sequence capable of modulating expression of a gene encoding a CCS52 protein is operably linked to a promoter that is other than a strong constitutive promoter. Advantageously, as illustrated in the examples, many promoters can be used for the methods of the present invention.

So, the promoters used in the present invention must have a different expression pattern than a strong constitutive promoter, such as the strong constitutive cauliflower mosaic virus 35S promoter (CaMV35S promoter). Having a different expression pattern means having the different expression level or having a different tissue-specificity or a combination of both. Preferably the promoter of the present invention is not a constitutive promoter. Constitutive means being expressed in most or in all of the tissues of the organism and independent from temporal regulation.

For example, a useful promoter for driving the CCS52 gene can have a strong expression level, but not in all or nearly all tissues so it is not constitutive. Preferably, strong promoters useful in the methods of the present invention for example are specific for a particular tissue such as for example the trichomes or the seeds. Also medium strength-tissue specific promoters are very useful for the present invention. According to preferred embodiment of the invention, the promoter used in the methods of the present invention is a tissue-specific promoter, for example a seed-specific.

According to another preferred embodiment of the invention, the promoter used in the methods of the present invention is a constitutive promoter other than a strong constitutive promoter. The term "constitutive" as defined herein refers to a promoter that is expressed predominantly in most or all tissue or organ and predominantly at any life stage of the plant. Preferably the promoter is expressed predominantly throughout the plant, thus has a ubiquitous expression.

The skilled craftsman will understand that a "constitutive promoter" is a promoter that is transcriptionally active throughout most, but not necessarily all parts of an organism, preferably a plant, during most, but not necessarily all phases of its growth and development. A low level of exprepression involved 0.5 or 1 molecule mRNA per cell. Generally, a mild or weak promoter is a promoter that drives expression of a coding sequence at a low level (at levels of about 1/10,000 transcripts to about 1/100,000 transcripts, to about 1/500,0000 transcripts). Conversely, a "strong promoter" drives expression of a coding sequence at high level, or at about 1/10 transcripts to about 1/100 transcripts to about 1/1,000 transcripts.

Further preferably, the constitutive promoter used in the methods of the present invention is a promoter which does not originate from a cauliflower mosaic virus, further preferably, which does not originate from mosaic virus, or from any other plant virus.

Preferably, promoters (constitutive or not) of medium strength are used for the methods of the present invention and are used to drive the expression of the CCS52 gene. Although proposed in the background art to use a strong constitutive promoter, such as the 35S promoter, use of such strong constitutive promoter to drive the expression of the CCS52 gene disrupts the normal growth and development of the plant. To the contrary the use of other promoters that are other than strong constitutive promoters lead to interesting phenotypic characteristics such as larger plants. Two promoters were used successfully to obtain the same improved growth characteristics, namely the constitutive sunflower ubiquitin and the seed-preferred 2S2 promoter that shows some weak expression in young plantlets due to leakiness.
Therefore, preferred examples of promoters other than strong constitutive promoters are, for example a constitutive sunflower ubiquitin promoter or the seed-preferred but leaky 2S2 promoter.

Thus, when a constitutive promoter is used, it should not have the same strong expression level as the 35S ptomoter.
When a strong promoter is used, it should not have the same constitutive/ubiquitous expression as the promoter. Preferably in this case the promoter should be specific for certain tissues.
As used herein promoters of medium strength are for example promoters that do not reach the same expression level in plant as the cauliflower mosaic 35S promoter. The 35S promoter is known to give high expression in a rather constitutive and a rather ubiquitous manner in various plant species. The expression pattern, i.e. the strength of a promoter and the tissue

specificity of a promoter can be measured by coupling the promoter to a reporter gene, such as GUS, and to measure the intensity of expression of the reporter gene by colorimetric quantification of the expression level, for example by a Beta-galactosidase staining.

Therefore, according to a preferred embodiment of the invention, there if provided a method for altering growth characteristics of a plant, such as increasing the size of the plant and/or increasing the size of plant organs, comprising expression of a CCS52 gene under the control of a promoter other than a strong constitutive promoter.

Further preferably, a preferred promoter as described above is a constitutive promoter that gives the similar expression pattern as the sunflower ubiquitin in *Arabidopsis*. Alternatively and/or additionally, a preferred promoter as described above is a promoter that gives the similar expression pattern as the *Arabidopsis* 2S2 promoter in *Arabidopsis*.

It is envisaged by the present invention that CCS52 overexpression according to the method of the present invention can be used also to alter growth characteristics of plants other than *Arabidopsis*. Therefore, also promoters from or active in other plants are useful as long as they are other than strong constitutive promoters. Examples of such promoters are constitutive promoters, such as GOS2, enolase promoter, TCTP promoter, fructose bi phosphate aldolase promoter, EIF1-4E promoter, EF1alpha promoter, FAD2 desaturase promoter, G3PDH promoter, or meristem specific promoters such as cyclin D3 or the cdc2a promoters or shoot specific promoters such as rubisco activase promoters or OPR1 promoters.

Therefore, according to further embodiments of the present invention there is provided a construct as described aboce, wherein said control sequences comprise at least a constitutive promoter, preferably a constitutive promoter that gives similar expression patterns as the sunflower ubiquitin promoter in Arabidopsis thaliana, further preferably the sunflower ubiquitin promoter or the promoter of the orthologous ubiquitin gene of another plant.

Further the invention provides a construct as mentioned above, wherein said control sequences comprise at least a tissue preferred promoter, preferably a tissue-preferred promoter that gives similar expression patterns as the seed-preferred Arabidopsis 2S2 promoter in Arabidopsis thaliana, further preferably a seed-preferred promoter with weak leaky expression the young shoots, most preferably the Arabidopsis 2S2 promoter or the promoter of the orthologous 2S2 gene of another plant.

Optionally, one or more terminator sequences may also be used in the construct introduced into a plant. The term "terminator" encompasses a control sequence which is a DNA sequence at the end of a transcriptional unit which signals 3' processing and polyadenylation of a primary transcript and termination of transcription. Additional regulatory elements may include transcriptional as well as translational enhancers. Those skilled in the art will be aware of terminator and enhancer sequences which may be suitable for use in performing the invention. Such sequences would be known or may readily be obtained by a person skilled in the art.

The genetic constructs of the invention may further include an origin of replication sequence which is required for maintenance and/or replication in a specific cell type. One example is when a genetic construct is required to be maintained in a bacterial cell as an episomal genetic element (e.g. plasmid or cosmid molecule). Preferred origins of replication include, but are not limited to, the f1-ori and colE1.

The genetic construct may optionally comprise a selectable marker gene. As used herein, the term "selectable marker gene" includes any gene which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a genetic construct of the invention. Suitable markers may be selected from markers that confer antibiotic or herbicide resistance. Cells containing the recombinant DNA will thus be able to survive in the presence of antibiotic or herbicide concentrations that kill untransformed cells. Examples of selectable marker genes include the *bar* gene which provides resistance to the herbicide Basta; the npt gene which confers resistance to the antibiotic kanamycin; the hpt gene which confers hygromycin resistance. Visual markers, such as the Green Fluorescent Protein (GFP) may also be used as selectable markers. An entire plant may be generated from a single transformed plant cell through cell culturing techniques known to those skilled in the art. Further examples of suitable selectable marker genes include the ampicillin resistance (Amp^r), tetracycline resistance gene (Tc^r), bacterial kanamycin resistance gene (Kan^r), phosphinothricin resistance gene, neomycin phosphotransferase gene (nptII), hygromycin resistance gene, β-glucuronidase (GUS) gene, chloramphenicol acetyltransferase (CAT) gene, green fluorescent protein (gfp) gene (Haseloff *et al.*, Proc. Natl. Acad. Sci. U.S.A, 94 (6), 2122-2127, 1997), and luciferase gene, amongst others.

The present invention also encompasses plants obtainable by the methods according to the present invention. The present invention therefore provides plants obtainable by the method according to the present invention, which plants have modified growth characteristics, such as increased plant size and/or increase plant organ size, and which plants have altered CCS52

protein activity and/or altered, preferably increased, expression levels of a nucleic acid sequence encoding a CCS52 protein.

According to a third embodiment of the present invention, there is provided a method for the production of transgenic plants having modified growth characteristics, such as increased plant size and/or increase plant organ size, comprising introduction-and-expression in a plant of a nucleic acid molecule of the invention.

More specifically, the present invention provides a method for the production of transgenic plants having modified growth characteristics, such as having larger plant size and/or larger organs, which method comprises:

(i) introducing into a plant or plant cell a nucleic acid sequence or a portion thereof encoding a CCS52 protein or a homologue, derivative or active fragment thereof, and/or introducing into a plant a construct according to any of claims 12 to 15,

(ii) cultivating the plant cell under conditions promoting regeneration and mature plant growth.

The protein itself and/or the nucleic acid itself may be introduced directly into a plant cell or into the plant itself (including introduction into a tissue, organ or any other part of the plant). According to a preferred feature of the present invention, the nucleic acid sequence is preferably introduced into a plant by transformation. The nucleic acid sequence is preferably as represented by SEQ ID NO: 1 or a portion thereof or sequences capable of hybridising therewith, or is a nucleic acid sequence encoding an amino acid sequence represented by SEQ ID NO: 2 or a homologue, derivative or active fragment thereof. Alternatively, the nucleic acid sequence is as represented by SEQ ID NO: 3 or 5 or a portion thereof, or sequences capable of hybridising therewith. The amino acid sequence may alternatively be represented by SEQ ID NO: 4 or 6 or by homologues, derivatives or active fragments of any of the aforementioned sequences.

The term "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention and a whole plant regenerated therefrom. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems),

and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem). The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid. Alternatively, it may be integrated into the host genome. The resulting transformed plant cell can then be used to regenerate a transformed plant in a manner known to persons skilled in the art.

Transformation of a plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the gene of interest into a suitable ancestor cell. Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. *et al.*, 1882, Nature 296, 72-74; Negrutiu I. *et al.*, June 1987, Plant Mol. Biol. 8, 363-373); electroporation of protoplasts (Shillito R.D. *et al.*, 1985 Bio/Technol 3, 1099-1102); microinjection into plant material (Crossway A. *et al.*, 1986, Mol. Gen Genet 202, 179-185); DNA or RNA-coated particle bombardment (Klein T.M. *et al.*, 1987, Nature 327, 70) infection with (non-integrative) viruses and the like. A preferred method according to the present invention is the protocol according to Hiei *et al.* 1994 in the case of rice transformation.

Generally after transformation, plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant.

Following DNA transfer and regeneration, putatively transformed plants may be evaluated, for instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively or additionally, expression levels of the newly introduced DNA may be monitored using Northern and/or Western analysis, both techniques being well known to persons having ordinary skill in the art.

The generated transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed to give homozygous second generation (or T2) transformants, and the T2 plants further propagated through classical breeding techniques.

The generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells

transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

According to a fourth embodiment of the present invention, there is provided plant cells, tissues, organs and whole plants that have been transformed or transfected with a gene construct of the invention and/or which exhibit one or more modified growth characteristics. The present invention clearly also extends to any plant cell or plant produced by any of the methods described herein, and to all plant parts and propagules thereof. The present invention extends further to encompass the progeny of a primary transformed or transfected cell, tissue, organ or whole plant that has been produced by any of the aforementioned methods, the only requirement being that progeny exhibit the same genotypic and/or phenotypic characteristic(s) as those produced in the parent by the methods according to the invention. The invention also includes host cells containing an isolated nucleic acid molecule encoding a protein capable of modulating a CCS52, preferably wherein the protein is a CCS52 protein. Preferred host cells according to the invention are plant cells. The invention also extends to harvestable parts of a plant such as but not limited to seeds, leaves, fruits, flowers, stem cultures, stems, rhizomes, tubers and bulbs.

The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The term "plant" also therefore encompasses suspension cultures, embryos, meristematic regions, callus tissue, leaves, seeds, roots, shoots, gametophytes, sporophytes, pollen, and microspores. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily *Viridiplantae*, in particular monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising *Acacia spp., Acer spp., Actinidia spp.,Aesculus spp., Agathis australis, Albizia amara, Alsophila tricolor, Andropogon spp., Arachis spp, Areca catechu, Astelia fragrans, Astragalus cicer, Baikiaea plurijuga, Betula spp., Brassica spp., Bruguiera gymnorrhiza, Burkea africana, Butea frondosa, Cadaba farinosa, Calliandra spp, Camellia sinensis, Canna indica, Capsicum spp., Cassia spp., Centroema pubescens, Chaenomeles spp.,Cinnamomum cassia, Coffea arabica, Colophospermum mopane, Coronillia varia, Cotoneaster serotina, Crataegus spp., Cucumis spp., Cupressus spp., Cyathea dealbata, Cydonia oblonga, Cryptomeria japonica, Cymbopogon spp., Cynthea dealbata, Cydonia oblonga, Dalbergia monetaria, Davallia divaricata, Desmodium spp., Dicksonia squarosa, Diheteropogon amplectens, Dioclea spp, Dolichos spp., Dorycnium rectum, Echinochloa pyramidalis, Ehrartia spp., Eleusine coracana, Eragrestis spp., Erythrina spp., Eucalyptus spp., Euclea schimperi, Eulalia villosa, Fagopyrum*

spp., Feijoa sellowiana, Fragaria spp., Flemingia spp, Freycinetia banksii, Geranium thunbergii, Ginkgo biloba, Glycine javanica, Gliricidia spp, Gossypium hirsutum, Grevillea spp., Guibourtia coleosperma, Hedysarum spp., Hemarthia altissima, Heteropogon contortus, Hordeum vulgare, Hyparrhenia rufa, Hypericum erectum, Hyperthelia dissoluta, Indigo incarnata, Iris spp., Leptarrhena pyrolifolia, Lespediza spp., Lettuca spp., Leucaena leucocephala, Loudetia simplex, Lotonus bainesii, Lotus spp., Macrotyloma axillare, Malus spp., Manihot esculenta, Medicago sativa, Metasequoia glyptostroboides, Musa sapientum, Nicotianum spp., Onobrychis spp., Omithopus spp., Oryza spp., Peltophorum africanum, Pennisetum spp., Persea gratissima, Petunia spp., Phaseolus spp., Phoenix canariensis, Phormium cookianum, Photinia spp., Picea glauca, Pinus spp., Pisum sativum, Podocarpus totara, Pogonarthria fleckii, Pogonarthria squarrosa, Populus spp., Prosopis cineraria, Pseudotsuga menziesii, Pterolobium stellatum, Pyrus communis, Quercus spp., Rhaphiolepsis umbellata, Rhopalostylis sapida, Rhus natalensis, Ribes grossularia, Ribes spp., Robinia pseudoacacia, Rosa spp., Rubus spp., Salix spp., Schyzachyrium sanguineum, Sciadopitys verticillata, Sequoia sempervirens, Sequoiadendron giganteum, Sorghum bicolor, Spinacia spp., Sporobolus fimbriatus, Stiburus alopecuroides, Stylosanthos humilis, Tadehagi spp, Taxodium distichum, Themeda triandra, Trifolium spp., Triticum spp., Tsuga heterophylla, Vaccinium spp., Vicia spp.Vitis vinifera, Watsonia pyramidata, Zantedeschia aethiopica, Zea mays, amaranth, artichoke, asparagus, broccoli, brussel sprout, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugarbeet, sugar cane, sunflower, tomato, squash tea, trees, grasses (including forage grass) and algae, amongst others. According to a preferred feature of the present invention, the plant is a monocotyledonous plant, further preferably a cereal, most preferably a plant selected from rice, maize (including forage corn), wheat, barley, soybean, sunflower, canola, sugarcane, alfalfa, millet, barley, rapeseed and cotton. Preferred plants to be used in the methods of the present invention are trees and forage crops such as for example forage grass and forage corn.

Therefore, according to preferred embodiments of the invention there are provided transgenic plants as mentioned above having altered growth characteristics, such as having larger size and/or larger organs, wherein said plant is a monocotyledonous plant, further preferably a cereal, most preferably a plant selected from grasses, rice, maize, wheat, barley, sugarcane, millet, barley; or wherein said plant is a dicotyledoneous plant, preferably a forage plant, most preferably a plant selected from sunflower, canola, soybean, medicago, alfalfa, rapeseed, clover and cotton or wherein said plant is a tree, preferably a harvestable tree, most preferably a tree selected from poplar, eucalyptus, pine,

Advantageously, performance of the method according to the present invention results in plants having a variety of modified growth characteristics, such modified growth characteristics including increased plant size, increased organ size, increased yield/biomass, modified cell division, modified endoreduplication, modified plant growth and architecture and a modified stress response, each relative to corresponding wild type plants. Advantageously, performance of the method according to the present invention results in plants having increased cell division and increased number of cells in combination with having larger cells. Accroding to one aspect of the invention, the larger cells are obtained by multiple endocycles and are therefore the result of endoreduplication. Normally endoreduplication involves the arrest in cell division. Now, according to a particular feature of the present invention, endoreduplication can be increased in combination with the increase in cell division. So far the use of CCS52 has been suggested to switch the cells from proliferation into differentiation (for example endoreduplication, Kondorosi et al. 1999). Here in the present invention it is shown for the first time how to use the CCS52 gene/protein to obtain a useful phenotype in a plant. This unique feature of the invention allows a person skilled in the art to modulate plant growth characteristics by endoreduplication with the extra bonus effect of simultaneously enhancing cell division, cell production and cell division rate. The combination of modulating multiple basic cellular processes simultaneously in one plant by introduction of one promoter-transgene cassette is unique, because there is not only a positive effect on all of these cellular processes but moreover there is a synergistic effect resulting in outstanding improvements of the plant characteristics such as for example the size of stems and leaves.

Therefore according to the invention, there is provided a method to improve plant growth characteristics, comprising increased expression in a plant of a nucleic acid sequence encoding a CCS52 protein and/or increased activity in a plant of a CCS52 protein.

Further there is provided a method to increase plant size and/or plant organ size, comprising increased expression in a plant of a nucleic acid sequence encoding a CCS52 protein and/or increased activity in a plant of a CCS52 protein.

The increase in organ size can be effected because of more cell division. Therefore the invention provides a method as mentioned above, wherein the increase of plant size and/or plant organ size is effected by increase in cell division

Alternatively and/or additionally, the increase in organ size can be effected because of higher rate of cell production. Therefore, the present invention also encompasses a method as

mentioned above, wherein said increase of plant size and/or plant organ size is effected by increase in rate of cell division.

Third the increase in organ size can be effected because of larger cells. For example larger cells maybe the result of enhanced endoreduplication. In the prior art it was taught that the CCSS52 product may switch proliferating cells to differentiation programs which, in case of endocycles, result in cell size increase. However in the present invention endoreduplication is promoted as well as proliferation.

Therefore the present invention also encompasses a method as mentioned above, wherein the increase of plant size and/or plant organ size is effected by endoreduplication.

It has now been shown in the present invention that the size of the stem, leaves and the whole plants can be increased and that the number of cells in the stems, leaves and flowers can be increased by overexpression of CCS52. More particularly it has been found in the present invention that in one plant endoreduplication is promoted, and simultaneously cell division is also promoted. The synergy of both improved cellular processes lead to an unexpected bonus effect and results favorable plant phenotypes such as larger size and bushier plant.

The term "increased yield" encompasses an increase in biomass in one or more parts of a plant relative to the biomass of corresponding wild-type plants. This may be manifested by an increase in, for example, leaf size and/or number and/or stem thickness and/or number etc. An increase in stem thickness may in turn lead to the plant having overall increased survivability due to, for example, increased wind/rain resistance. An increase in the number of stems (shoots) will also contribute to increased branching, which in turn may contribute to increased bushiness of a plant. The term also encompasses an increase in seed yield, which includes an increase in the biomass of the seed (seed weight) and/or an increase in the number of (filled) seeds and/or in the size of the seeds and/or an increase in seed volume, each relative to corresponding wild-type plants. An increase in seed size and/or volume may also influence the composition of seeds. An increase in seed yield could be due to an increase in the number and/or size of flowers. An increase in yield might also increase the harvest index, which is expressed as a ratio of the total biomass over the yield of harvestable parts, such as seeds. An increase in yield also encompasses a better performance of the plant under non-stress conditions or under stress conditions compared to wild-type plants. Stress conditions include any type of environmental stress and biotic and abiotic stresses.

According to a preferred feature of the present invention, performance of the methods according to the present invention result in plants having modified yield. Preferably, the modified yield is an increase in aboveground biomass. Therefore, according to the present invention, there is provided a method for increasing yield, which method comprises modulating expression of a nucleic acid sequence encoding a CCS52 protein and/or modulating activity of a CCS52 protein in a plant, preferably wherein the CCS52 protein is encoded by a nucleic acid sequence represented by SEQ ID NO: 1 or a portion thereof or sequences capable of hybridising therewith or wherein the CCS52 protein is represented by SEQ ID NO: 2 or a homologue, derivative or active fragment thereof. Alternatively, the CCS52 protein may be encoded by a nucleic acid sequence represented by SEQ ID NO: 3 or 5 or by a portion thereof, or by sequences capable of hybridising therewith, or wherein the CCS52 protein is represented by SEQ ID NO: 4 or 6 or a homologue, derivative or active fragment thereof.

The term "modified cell division" encompasses an increase or decrease in cell division or an abnormal cell division/cytokinesis, altered plane of division, altered cell polarity, altered cell differentiation. Modified cell division may also give rise to altered cell size and cell number. Upon overexpression of a gene encoding a CCS52 protein an increase in number of cells has been observed due to increased cell divions and/or increased cell division rate.

Enlarged cells will also have thinner surrounding cell walls which in turn will render the plant in question more digestible. This can have useful applications in, for example, forage crops. This will also be useful in trees, ornamentals, to increase biomass, or change architecture

The term "modified endoreduplication" as defined herein means the recurrent replication of DNA without subsequent mitosis and cytokinesis. Endoreduplication leads to increased DNA accumulation which in turn regulate cell size.

The term "modified plant growth" as used herein encompasses, but is not limited to, a faster rate of growth in one or more parts of a plant (including seeds), at one or more stages in the life cycle of a plant, and/or enhanced vigour, each relative to corresponding wild-type plants. Increased growth rate may therefore lead to early flowering. An increase in growth rate may also alter the harvest time of a plant allowing plants to be harvested sooner than would otherwise be possible. If the growth rate is sufficiently increased, it may even give rise to the possibility of the early collecting of seeds (if there is early flowering) or of green biomass. It may give the possibility of the early sowing of a second generation of the same plant species (for example sowing and harvesting of sugarcace followed by sowing and harvesting of further sugarcane all within one conventional growing period) or of different plants species (for

example the sowing and harvesting of sugarcane followed by, for example, the sowing and optional harvesting of soy bean, grasses, potatoes or any other suitable plant), thereby increasing the annual biomass production per acre (due to an increase in the number of times (say in a year) that any particular plant may be grown and harvested).

Therefore, according to the present invention, there is provided a method for increasing plant growth, which method comprises modulating expression of a nucleic acid sequence encoding a CCS52 protein and/or modulating activity of a CCS52 protein in a plant, preferably wherein the CCS52 protein is encoded by a nucleic acid sequence represented by SEQ ID NO: 1 or a portion thereof or sequences capable of hybridising therewith or wherein the CCS52 protein is represented by SEQ ID NO: 2 or a homologue, derivative or active fragment thereof. Alternatively, the CCS52 protein may be encoded by a nucleic acid sequence represented by SEQ ID NO: 3 or 5 or by a portion thereof, or by sequences capable of hybridising therewith, or wherein the CCS52 protein is represented by SEQ ID NO: 4 or 6 or a homologue, derivative or active fragment thereof.

The aforementioned attributes are of great agricultural and horticultural importance since increased growth reduces the time-to-harvest of crop plants. This improvement is also of considerable value in the case of forage crops. Furthermore, increased branching and increased plant vigor can be expected to have flow-on effects in terms of increased floral initiation, increased fruiting and higher seed yields. Such improvements are clearly of value to humans because seed proteins are of high nutritional value to both humans and livestock.

The present invention also relates to use of a nucleic acid sequence encoding a CCS52 protein and homologues, derivatives and active fragments thereof in modifying the growth characteristics of plants, preferably in increasing yield, further preferably seed yield. The present invention also relates to use of a CCS52 protein and homologues, derivatives and active fragments thereof in modifying the growth characteristics of plants. The nucleic acid sequence is preferably as represented by SEQ ID NO: 1 or a portion thereof or sequences capable of hybridising therewith or is an amino acid sequence represented by SEQ ID NO: 2 or a homologue, derivative or active fragment thereof.

"Modified architecture" may be due to change in cell division. The term "architecture" as used herein encompasses the appearance or morphology of a plant, including any one or more structural features or combination of structural features thereof. Such structural features include the shape, size, number, position, texture, arrangement, and pattern of any cell, tissue or organ or groups of cells, tissues or organs of a plant, including the root, leaf, shoot, stem,

petiole, trichome, flower, inflorescence (for monocots and dicots), panicles, petal, stigma, style, stamen, pollen, ovule, seed, embryo, endosperm, seed coat, aleurone, fibre, cambium, wood, heartwood, parenchyma, aerenchyma, sieve element, phloem or vascular tissue, amongst others. Modified architecture therefore includes all aspects of modified growth of the plant. Sometimes plants modify their architecture in response to certain conditions such as stress and pathogens (e.g. nematodes).

According to a preferred feature of the present invention, the term "modified architecture" encompasses, but is not limited to the following characteristics

- a change in the morphology of the leaves, such as area, thickness, shape etc.;
- Modified cell form, for example, modified cell size and/or shape;
- Modified epidermal outgrowth (e.g. trichome) structures, such as modified size of the epidermal outgrowth, modified number of branches, modified content etc. An increased production of specialised epidermal outgrowth structures, such as cotton fibres or glandulars trichomes, would have various economic and other advantages. Specialised trichomes could also be used for the production of useful metabolites, pharmaceutical compounds, nutraceuticals and food additives.
- modified stems, such as increased thickness of the stems, increased number of stems etc..

These improved growth characteristics give rise to increased branching, which is important in for example tree and sugar cane cultivation. These improved growth characteristics give also rise to a bushier plant phenotype, which is important in for example grass cultivation. A bushier phenotype also encompasses advantages in conferring increased aesthetic value in ornamentals, increased value in all crops due to increased flowers and/or fruits, conferring more efficient harvesting for example due to better accessibility of harvesting machinery, and to confer to the plant improved ability to withstand adverse environmental conditions. The stems may also have modified architecture, including for example different arrangements of lateral stems, a different shape, different arrangement of the flowers and the fruits. The flatter characteristic may even improve the harvest of the fruits and/or flowers).

These changes in morphology are also particularly useful when the plant grows under stress conditions. For example modifications of the architecture of the leaves, such as the leaf thickness has a pronounced effect on the tolerance of the plant against stress conditions, such as against drought stress. Increasing branching of epidermal outgrowths also increases the defense of a plant against outside threats. Increasing the number and/or the branching of trichomes may decrease the susceptibility of the leaves to insect colonization. Further increasing branching of epidermal outgrowths increasing stress tolerance by decreased

transpiration. The evaporation in the microlayer of air trapped in the trichome layer will delay or impede temperature increase and will limit transpiration. Trichomes can create microshadows on the leaves. Increasing trichome branching, increasing trichome surface per leaf will create the capacity to produce high value products and harvest high value molecules from the biomass produced per plant.

In particular circumstances also spines on epidermal outgrowths may serve an added function of modifying the plant body temperature. For example in certain cacti, where a spine canopy keeps a shoot apex a degree or two warmer on a cold night or reflects some infrared radiation during the day, cooling it slightly.

The present invention also relates to use of a nucleic acid sequence encoding a CCS52 protein and homologues, derivatives and active fragments thereof and to the CCS52 protein itself and to homologues, derivatives and active fragments thereof as a growth regulator. The sequences represented by SEQ ID NO: 1 and portions thereof and SEQ ID NO: 2 and homologues, derivatives and active fragments thereof are useful in modifying the growth characteristics of plants, as hereinbefore described. The sequences would therefore find use as growth regulators, such as herbicides or growth stimulators. The present invention also provides a composition comprising a protein represented by SEQ ID NO 2 or a homologue, derivative or active fragment thereof for the use as a growth regulator.

Conversely, the sequences according to the present invention may also be interesting targets for agrochemical compounds, such as herbicides or growth stimulators. Accordingly, the present invention encompasses use of a nucleic acid sequence as represented by SEQ ID NO: 1 or a portion thereof or a sequence represented by SEQ ID NO: 2 or homologues, derivatives and active fragments thereof as targets for an agrochemical compound, such as a herbicide or a growth stimulator.

The methods of the present invention lead to modified activity of the CCS52 protein in the plant cell, which CCS52 protein is known to be involved function/activation of the APC complex. Therefore it is assumed that modifying the CCS52 protein activity will affect the function/activation of the APC complex, its role in protein degradation for example degradation of cell cycle genes such as cyclins, the segregation of chromosomes during mitosis and its role in cell division.

The methods according to the present invention may also be practised by co-expression of a gene encoding a CCS52 protein in a plant with at least one other gene that cooperates with

the gene encoding a CCS52 protein. Such a gene may be any other CCS52 gene (which may be a CCS52A gene or a CCS52B gene or a CDC20 gene or any component of the APC complex. Co-expression may be effected by cloning the genes under the control of a plant expressible promoter in a plant expressible vector and introducing the expression vector(s) into a plant cell using *Agrobacterium*-mediated plant transformation.

The methods according to the present invention result in plants having modified growth characteristics, as described hereinbefore. These advantageous growth characteristics may also be combined with other economically advantageous traits, such as further yield-enhancing traits, tolerance to various stresses, traits modifying various architectural features and/or biochemical and/or physiological features.

The present invention will now be described with reference to the following figures in which:

Fig. 1 is a map of the entry clone, p1627, containing the gene of interest, CCS52A1, (internal reference CDS0198) within the AttL1 and AttL2 sites for Gateway® cloning in the pDONR201 backbone. This vector also contains a bacterial kanamycine-resistance cassette and a bacterial origin of replication.

Fig. 2 is a map of the binary vector for expression in *Arabidopsis thaliana* of the *Arabidopsis thaliana* CCS52A1 gene (CDS0198) under the control of the UBIQUITIN promoter (internal reference PRO155). This vector contains a T-DNA derived from the Ti Plasmid, limited by a left border (LB repeat, LB Ti C58) and a right border (RB repeat, RB Ti C58)). From the left border to the right border, this T-DNA contains: a cassette for antibiotic selection of transformed plants; a CaMV35S promoter – GFP CDS – NOS terminator cassette for visual screening of transformed plants; the PRO155 - CDS0198 -zein and rbcS-deltaGA double terminator cassette for expression of the *Arabidopsis thaliana* CCS52A1 gene. This vector also contains an origin of replication from pBR322 for bacterial replication and a selectable marker (Spe/SmeR) for bacterial selection with spectinomycin and streptomycin.

Fig. 3 shows an aerial view of a transgenic *Arabidopsis* plant expressing a CCS52A1 gene under the control of an ubiquitin promoter (transgenic) and a corresponding wild type plant, both of which are 4 weeks.

Fig. 4 shows a first cauline leaf of a transgenic *Arabidopsis* plant expressing a CCS52A1 gene under the control of an ubiquitin promoter and the first cauline leaf of a corresponding wild type plant. As shown, the leaf of the transgenic plant is of a different shape and of a larger size than the corresponding wild type plant.

Fig. 5 shows a first rosette leaf of a transgenic *Arabidopsis* plant expressing a CCS52A1 gene under the control of an ubiquitin promoter and a first rosette leaf of a corresponding wild type plant. As shown, the rosette leaf of the transgenic plant has increased width and a larger area than the corresponding wild type plant. Also noticeable, is an intrinsic increase in the vascularisation system of the leaf of the transgenic plant expressing CCS52A1 gene under the control of an ubiquitin promoter.

Fig. 6 shows leaf tissues of a transgenic *Arabidopsis* plant expressing a CCS52A1 gene under the control of an ubiquitin promoter and of leaf tissues of a corresponding wild type plant. As shown, the cell size of the transgenic plant is bigger than that of the control wild type plant.

Fig. 7 shows photographs of epidermis and trichomes of a transgenic Arabidopsis plant (Fig. 7a) expressing a CCS52A1 gene under the control of an ubiquitin promoter and of epidermis and trichomes of a corresponding wild type plant (Fig. 7b). As shown the trichomes of transgenic plant are more branched than that of the control wild type plant.

Fig. 8 shows a transgenic *Arabidposis* plant expressing a CCS52A1 gene under the control of a 2S2 promoter and a corresponding wild type plant. As shown, the transgenic plant has a greater number of leaves and has a bushier phenotype, has at least 2 times the number of rosette branches, has more flowers and more lateral branches.

Fig. 9 shows a transgenic *Arabidposis* plant expressing a CCS52A1 gene under the control of an ubiquitin promoter and a corresponding wild type plant. As shown, the diameter of the main stem and of the rosette and lateral branches is increased in transgenic plants.

Fig. 10 shows transversal sections of stem of transgenic *Arabidopsis* plant expressing a CCS52A1 gene under the control of an ubiquitin promoter and a corresponding wild type plant. As shown, the stem section is enlarged in the transgenic plant. In stems there is a 3-fold increase in the number of cells when the plants are transformed with CCS52A1.

Fig. 11 shows seeds produced by transgenic *Arabidopsis* plant expressing a CCS52A1 gene under the control of an ubiquitin promoter and a corresponding wild type plant. As shown, seed size is enlarged in the transgenic plant.

Fig. 12 shows a phylogentic tree of CCS52 related proteins in plants and animals. The proteins are presented by their Genbank accession number. Multiple-sequence alignment was done using CLUSTAL W (Higgins et al., (1994). CLUSTAL W: improving the sensitivity of progressivemultiple sequence alignment through sequence weighting,position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680). Sequences were input in a FASTA format and were aligned across their whole sequence. Default parameters were used for the alignment. The matrix used to generate the multiple sequence alignment was BLOSSUM 62, GAPOPEN (penalty for opening a gap) was used at the default value is 10, ENDGAP (penalty for closing a gap) was not used, GAPEXT (penalty for extending a gap) was used at the default value is 0.05, and GAPDIST (gap separation penalty) was used at the default value is 8.

For the construction and viewing of the tree, we choose Phylogram that is a branching diagram (tree) assumed to be an estimate of a phylogeny, branch lengths are proportional to the amount of inferred evolutionary change.

Fig 13 shows the conserved consensus motifs in plant CCS52 related proteins.

Fig.14 shows the sequences of the present invention with their respective SEQ ID numbers

Fig. 15 is a map of the binary vector p3965 for expression in *Arabidopsis thaliana* of the *Arabidopsis thaliana* CCS52A1 gene (CDS0198) under the control of the 35S promoter. This vector contains a T-DNA derived from the Ti Plasmid, limited by a left border (LB repeat, LB Ti C58) and a right border (RB repeat, RB Ti C58)). From the left border to the right border, this T-DNA contains: a cassette for antibiotic selection of transformed plants; a CaMV35S promoter – GFP CDS – NOS terminator cassette for visual screening of transformed plants; the 35S - CDS0198 -zein and rbcS-deltaGA double terminator cassette for expression of the *Arabidopsis thaliana* CCS52A1 gene. This vector also contains an origin of replication from pBR322 for bacterial replication and a selectable marker (Spe/SmeR) for bacterial selection with spectinomycin and streptomycin.

Fig 16. shows wild type *Arabidopsis thaliana* plants and transgenic *Arabidopsis thaliana* plants transformed with the vector p 3965 carrying the 35S-AtCCS52A1 expression cassette.

Examples

The present invention will now be described with reference to the following examples, which are by way of illustration alone.

DNA Manipulation

Unless otherwise stated, recombinant DNA techniques are performed according to standard protocols described in (Sambrook (2001) Molecular Cloning: a laboratory manual, 3rd Edition Cold Spring Harbor Laboratory Press, CSH, New York) or in Volumes 1 and 2 of Ausubel *et al.* (1984), Current Protocols in Molecular Biology, Current Protocols. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfase (1993) by R.D.D. Croy, published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications (UK).

Example 1: Gene Cloning

The *Arabidopsis* CCS52A1 (CDS0198) was amplified by PCR using as template an *Arabidopsis thaliana* seedling cDNA library (Invitrogen, Paisley, UK). After reverse transcription of RNA extracted from seedlings, the cDNAs were cloned into pCMV Sport 6.0. Average insert size of the bank was 1.5 kb, and original number of clones was of $1.59x10^7$ cfu. Original titer was determined to be $9.6x10^5$ cfu/ml, after first amplification of $6x10^{11}$ cfu/ml. After plasmid extraction, 200 ng of template was used in a 50 µl PCR mix. Primers prm01391 (sense, start codon in bold, AttB1 site in italic: 5' *GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACA* **ATG**GAAGAAGAAGATCCTACAGC 3') and prm01392 (reverse, complementary, AttB2 site in italic: 5' *GGGGACCACTTTGTACAAGAAAGCTGGGT* TTCTCACCGAATTGTTGTTCTAC 3'), which include the AttB sites for Gateway recombination, were used for PCR amplification. PCR was performed using Hifi Taq DNA polymerase in standard conditions. A PCR fragment of 1557 bp was amplified and purified also using standard methods. The first step of the Gateway procedure, the BP reaction, was then performed, during which the PCR fragment recombines in vivo with the pDONR201 plasmid to produce, according to the Gateway terminology, an "entry clone", p1627 (Figure 1). Plasmid pDONR201 was purchased from Invitrogen, as part of the Gateway® technology.

Example 2: Vector construction

The entry clone p1627 was subsequently used in an LR reaction with p0712, a destination vector used for *Arabidopsis thaliana* transformation. This vector contains as functional elements within the T-DNA borders: a plant selectable marker; a GFP expression cassette; and a Gateway cassette intended for LR *in vivo* recombination with the sequence of interest already cloned in the entry clone. A UBIQUITIN promoter for constitutive expression (PRO155)

is located upstream of this Gateway cassette. After the LR recombination step, the resulting expression vector p1652 (Figure 2) was transformed into *Agrobacterium* strain LBA4044 and subsequently to *Arabidopsis thaliana* plants.

Example 3: Arabidopsis transformation

Sowing and growing of the parental plants

For the parental plants, approximately 12 mg of wild-type *Arabidopsis thaliana* (ecotype Columbia) seeds were suspended in 27.5 ml of 0.2 % agar solution. The seeds were incubated for 2 to 3 days at a temperature of 4°C and then sown. The plants were germinated under the following standard conditions: 22°C during the day, 18°C at night, 65-70% relative humidity, 12 hours of photoperiod, sub-irrigation with water for 15 min every 2 to 3 days. The seedlings that developed were then transplanted to pots with a diameter of 5.5cm, containing a mixture of sand and peat in a ratio of 1 to 3. The plants were then further grown under the same standard conditions as mentioned above.

Agrobacterium growth conditions and preparation

Agrobacterium strain C58C1RIF with helper plasmid pMP90 containing vector p1652 was inoculated in a 50 ml plastic tube containing 1 ml LB (Luria Broth) without antibiotic. The culture was shaken for 8-9h at 28°C. Following this, 10 ml of LB without antibiotic was added to the plastic tube and shaken overnight at 28°C. Following this, the OD at 600 nm was measured. At an optical density of approximately 2.0, 40 ml of 10% sucrose and 0.05% Silwet L-77 (a chemical mixture of polyalkyleneoxide modified heptamethyltrisiloxane (84%) and allyloxypolyethyleneglycol methyl ether (16%), OSI Specialties Inc) was added to the culture. The *Agrobacterium* culture obtained was labelled CD2175 and used to transform the grown plants.

Flower dip

When each parental flower had one inflorescence of 7-10 cm in height, the inflorescences were inverted into the *Agrobacterium* culture and agitated gently for 2-3 seconds. 2 plants per transformation were used. Following this, the plants were returned to the normal growing conditions as described above.

Seed collection

5 weeks after the flowers were dipped in the *Agrobacterium* culture, watering of the plants was stopped. The plants were incubated at 25°C with a photoperiod of 20 hours. One week later, the seeds were harvested and placed in a seed drier for one week. The seeds were then

cleaned and collected in 15 ml plastic tubes. The seeds were then stored at 4°C until further processing.

Example 4: Evaluation of transgenic plants

Selection of the first generation of transgenic plants

100mg of seeds were placed in a 50ml plastic tube. 27ml of a 0.2% agar solution was added and mixed to suspend the seeds. The tubes were stored at 4° C for 3 days to release the seeds from dormancy. Following this period, the seed suspension was examined under blue light to determine the presence of transformed seeds. 20 bright fluorescent seeds (expressing the transgene) were aspirated with a Pasteur pipette, transferred to a 15ml plastic tube, and the suspension volume was adjusted to 15ml with 0.2% agar. The same amount of non-fluorescent seeds (not expressing the transgene) was transferred to a separate 15ml plastic tube and the suspension volume adjusted to 15ml with 0.2% agar. The suspension of expressing seeds was evenly dispensed as drops of 50µl on one half of a 50x30cm tray containing a mixture of sand and soil in a ratio of 1 to 2. The non-expressing seeds were dispensed in the same way on the other half of the tray. The tray was placed in a greenhouse under the following conditions: 22°C during the day, 18°C at night, 60% relative humidity, 20 hour photoperiod, sub-irrigation once a day with water for 15 min. On the 14th day after sowing, 5 expressing and 5 non-expressing seedlings were transplanted into individual pots with a diameter of 10cm containing a mixture of sand and peat in a ratio of 1 to 3.

Cultivation and imaging of the first generation of transgenic plants

The pots were then placed in a greenhouse under the same conditions as described for the trays. The pots were sub-irrigated for 15 minutes, once a week, or more if needed. On the 21st, 28th, 35th, 42nd and 49th day after sowing, the rosettes of each plant were photographed using a digital camera. On the 35th, 42nd, 49th and 56th day after sowing, the inflorescence of each plant was photographed, also using a digital camera. The number of pixels corresponding to plant tissues was recorded on each picture, converted to cm^2 and used as a measurement of plant size. On the 57th day after sowing, when the first siliques were ripening, a breathable plastic bag was placed on each plant and tightly attached at the base of the plants to collect the shedding seeds. On the 90th day after sowing, when all the siliques were ripe, the seeds were collected and placed in a seed drier for 1 week before storage in a sealed container at 4°C.

Seed yield of the first generation of transgenic plants

Harvested inflorescences of the T1 plants were taken and gently rubbed to release seeds from the siliques. The mixture of seeds and chaff was then passed over a mesh to remove large fragments of stems, leaves, siliques, etc. The seeds were then poured onto a vibrating gutter

equipped with a vacuum cleaner allowing the lighter fragments, such as petals and small fibers, to be aspirated whilst retaining the heavier seeds. Data on the seed parameters were measured using an automated system.

A similar procedure was followed to evaluate the phenotypic characteristics of *Arabidopsis* T2 lines. 15 expressing and 15 non-expressing seedlings were transplanted into individual pots with a diameter of 10cm (containing a mixture of sand and peat in a ratio of 1 to 3) and processed as described above.

Example 5: Phenotypic characteristics of transgenic plants

Increased biomass

Transgenic plants showed increased biomass relative to control plants. This was manifested by an increase in leaf size (see Fig. 4 and 5), an increase in the number of rosette leaves (see Fig. 3), and increase in the number of cauline leaves, an increase in stem thickness (see Fig. 9 and 10) , more branching, giving rise to a bushy phenotype (see Fig. 9). As a consequence it is estimated that overall plant biomass is multiplied by 3 to 4 in transgenic *Arabidopsis* plants.

Modified trichomes

Transgenic plants showed trichomes with increased number of branches when compared to the control plants (see Fig. 7).

Example 6: Overexpression of CCS52 under control of the cauliflower mosaic virus 35S promoter in Arabidopsis results in small, aberrant plants

The AtCCS52A1 gene was cloned from the entry clone p1627 via a Gateway LR reaction with a destination vector used for *Arabidopsis thaliana* transformation and carrying the 35S promoter. This vector contains as functional elements within the T-DNA borders: a plant selectable marker; a GFP expression cassette; and a Gateway cassette intended for LR *in vivo* recombination with the sequence of interest already cloned in the entry clone. A 35S promoter for strong constitutive expression is located upstream of this Gateway cassette. After the LR recombination step, the resulting expression vector p3965 (Figure 15) was transformed into *Agrobacterium* strain LBA4044 and subsequently to *Arabidopsis thaliana* plants.

Arabidopsis plant were regenerated and grown under optimal growth conditions as mentioned above. Nullizygote plant without the transgene were alternated with transgenic plant comprising the transgene in a growing tray (figure 16). During growth in optimal conditions, a significant difference between transgenic and wild-type plant could be observed. After 5 to 6 weeks the plants were photographed (figure 16). At this stage the transgenic plants showed a

small and aberrant phenotype compared with the mature and healthy wild type plant. The transgenic plant clearly had smaller leaves, smaller or no stems, smaller rosette diameter, less leaves, less flowers. Clearly these plants suffer from an early growth arrest. In transgenic plants the leaved are reddish, indicating that these plant suffer from stress. The plant further produces throughout its entire lifespan significantly reduced amounts of siliques and seeds, compared to the wild-type plants.

Example 7: Overexpression of the rice CCS52 gene under control of the pGOS, oleosin promoter or prolamin promoter, in Oryza sativa results in larger plants

The OsCCS52A gene (AAM74839, SEQ ID NO 3, with corresponding protein SEQ ID NO 4) is cloned in a Gateway entry clone and subsequently cloned via a LR reaction with a destination vector used for Oryza staiva transformation and carrying the pGOS promoter or the prolamin promoter or the oleosin promoter. After the LR recombination step, the resulting expression vector is transformed into *Agrobacterium* strain LBA4044 and subsequently to Oryza sativa plants following the transformaion p^rotocol of Hiei et al. (Plant J., 6 (2), 271-282, 1994).
The transgenic plants have enhanced biomass, enhanced number of tillers, enhanced stem diameter, enhanced leave number, enhanced seed biomass, and early flowering, compared to the corresponding nullizygots.

Claims

1. Method to improve plant growth characteristics, comprising increased expression in a plant of a nucleic acid sequence encoding a CCS52 protein and/or increased activity in a plant of a CCS52 protein.

2. Method according to claim 1 to increase plant size and/or plant organ size, comprising increased expression in a plant of a nucleic acid sequence encoding a CCS52 protein and/or modified activity in a plant of a CCS52 protein.

3. Method according to any of claim 1 or 2, wherein said increase of plant size and/or plant organ size is effected by increase in cell division

4. Method according to any of claim 1 or 3, wherein said increase of plant size and/or plant organ size is effected by increase in rate of cell division.

5. Method according to any of claims 1 to 4, wherein said increase of plant size and/or plant organ size is effected by endoreduplication.

6. Method according to any of claims 1 to 5, wherein said increase in expression and/or activity is effected by recombinant means and/or chemical means.

7. Method according to any of claims 1 to 6, wherein said increased expression comprises introducing into a plant a nucleic acid sequence encoding a CCS52 protein or a homologue, derivative or active fragment thereof.

8. Method according to claim 7 wherein said nucleic acid is a homologous nucleic acid sequence, preferably from rice, further preferably is a nucleic acid as represented by any of SEQ ID NO: 1, 3 or 5 or a portion thereof or sequences capable of hybridising therewith, or a nucleic acid sequence encoding an amino acid sequence represented by any of SEQ ID NO: 2, 4, 6 or a homologue, derivative or active fragment thereof.

9. Method according to any of claims 1 to 8, wherein said nucleic acid sequence encoding a CCS52 protein is overexpressed in a plant.

10. Method according to any of claims 1 to 9, wherein expression of said nucleic acid encoding a CCS52 protein is driven by a promoter other than a strong constitutive promoter.

11. Method according to any of claims 1 to 10, wherein said plant organ is selected from any one or more of leaf, stem, stem culture, flower, seed, root, fruit, rhizome, tuber, bulb.

12. Plants or plant organs obtainable by a method according to any of claims 1 to 11.

13. Genetic construct comprising:

(a) a nucleic acid sequence capable of modulating expression of a nucleic acid encoding a CCS52 protein and/or activity of a CCS52 protein;

(b) one or more control sequence capable of driving expression of the nucleic acid sequence of (i) said sequence being other than a strong constitutive promoter; and optionally

(c) a transcription termination sequence, and

(d) wherein said control sequence of (b) is not the *endod12Ams* or the *Srglb3* promoter.

14. Construct according to claim 13, wherein said nucleic acid sequence capable of modulating expression of a nucleic acid encoding a CCS52 protein and/or activity of a CCS52 protein is a nucleic acid sequence encoding a CCS52 protein, preferably as represented by SEQ ID NO: 1, 3 or 5 or a portion thereof or by sequences capable of hybridising therewith or a nucleic acid sequence encoding an amino acid sequence represented by any of SEQ ID NO: 2, 4, 6 or a homologue, derivative or active fragment thereof.

15. Construct according to claim 13 or 14 wherein said control sequence being other than a strong constitutive promoter is a promoter of medium strength and wherein said control sequence of is not the *endod12Ams* or the *Srglb3* promoter.

16. Construct according to claim 13 or 15, wherein said control sequences comprise at least a constitutive promoter, preferably a constitutive promoter that gives similar expression patterns as the sunflower ubiquitin promoter in Arabidopsis thaliana, further

preferably the sunflower ubiquitin promoter or the promoter of the orthologous ubiquitin gene of another plant.

17. Construct according to claim 13 or 15, wherein said control sequences comprise at least a tissue preferred promoter, preferably a tissue-preferred promoter that gives similar expression patterns as the seed-preferred Arabidopsis 2S2 promoter in Arabidopsis thaliana, further preferably a seed-preferred promoter with weak leaky expression the young shoots, most preferably the Arabidopsis 2S2 promoter or the promoter of the orthologous 2S2 gene of another plant.

18. Method for the production of a transgenic plant having larger size and/or larger organs, which method comprises:
    a) introducing into a plant or plant cell a nucleic acid sequence or a portion thereof encoding a CCS52 protein or a homologue, derivative or active fragment thereof; and/or introducing into a plant a construct according to any of claims 13 to 16,
    b) cultivating the plant cell under conditions promoting regeneration and mature plant growth.

19. Transgenic plant having larger size and/or having larger organs, characterised in that said plant has modulated expression in a plant of a nucleic acid sequence encoding a CCS52 protein and/or modulated activity in a plant of a CCS52 protein.

20. Transgenic plant according to claim 19, wherein said plant is a monocotyledonous plant, further preferably a cereal, most preferably a plant selected from grasses, rice, maize, wheat, barley, sugarcane, millet, barley,.

21. A transgenic plant according to claim 19 wherein said plant is a dicotyledoneous plant, preferably a forage plant, most preferably a plant selected from sunflower, canola, soybean, medicago, alfalfa, rapeseed, clover and cotton ...

22. A transgenic plant according to claim 19, wherein said plant is a tree, preferably a harvestable tree, most preferably a tree selected from poplar, eucalyptus, pine, ...

23. Use of a nucleic acid sequence encoding a CCS52 protein and homologues, derivatives and active fragments thereof for increasing the size of plants or plant organs.

24. Use of a CCS52 protein and homologues, derivatives and active fragments thereof for increasing the size of plants or the size of plant organs.

25. A composition comprising a protein represented by SEQ ID NO 2, 4 or 6 or a homologue, derivative or active fragment thereof for the use as a growth regulator.

26. Use of a nucleic acid sequence as represented by SEQ ID NO: 1, 3 or 5 or a homologue or a portion thereof or a protein represented by SEQ ID NO: 2, 4 or 6 or homologues, derivatives and active fragments thereof as a target for an agrochemical compound, such as a herbicide or a growth stimulator.

Abstract

Plants having improved growth characteristics and a method for making the same

The present invention concerns a method for improving the growth characteristics of plants by modulating expression in a plant of a nucleic acid sequence encoding a CCS52 protein and/or modulating activity in a plant of a CCS52 protein. The invention also relates to transgenic plants having improved growth characteristics, such as larger plants or plants with more and/or larger organs, which plants have modulated expression of a nucleic acid encoding a CCS52 protein.

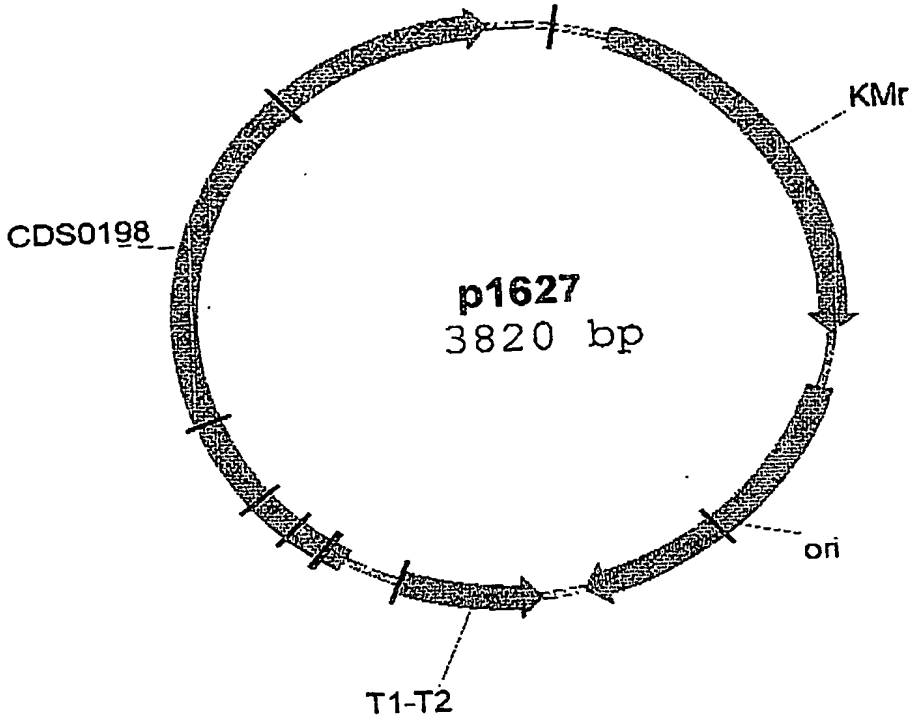


FIGURE 1

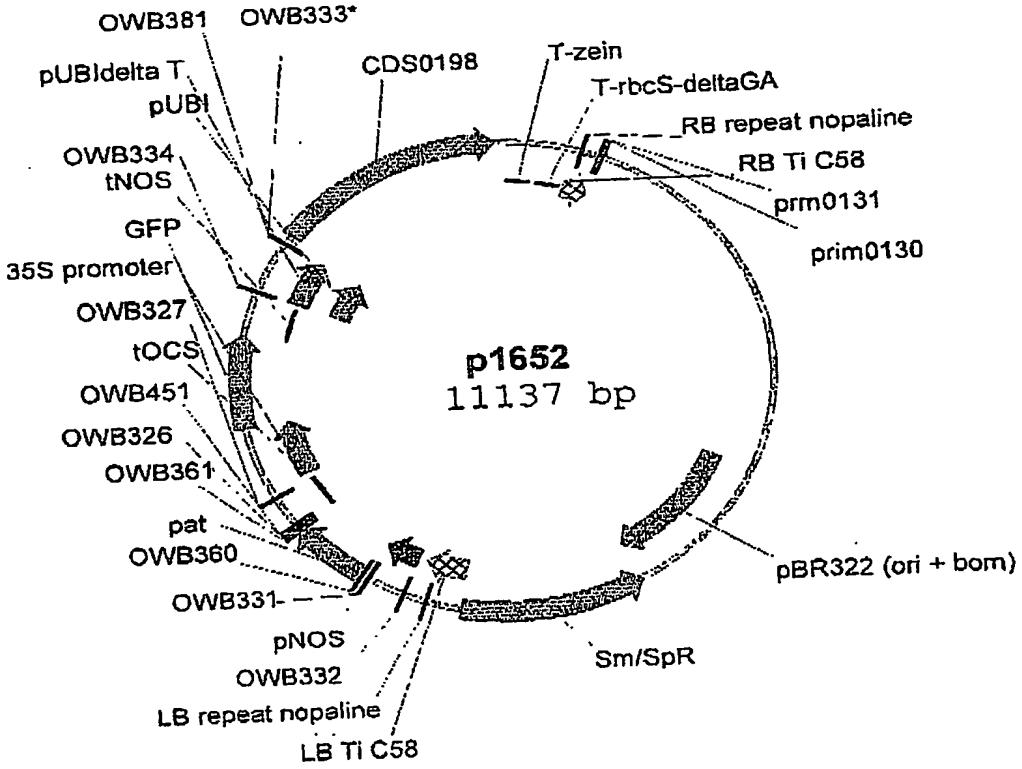
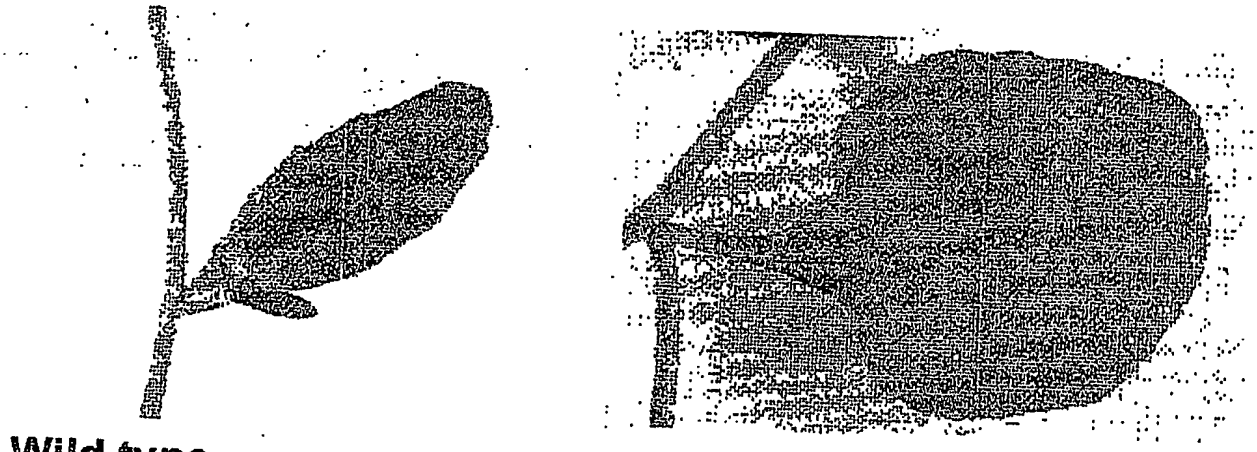


FIGURE 2

Wild type

Transgenic

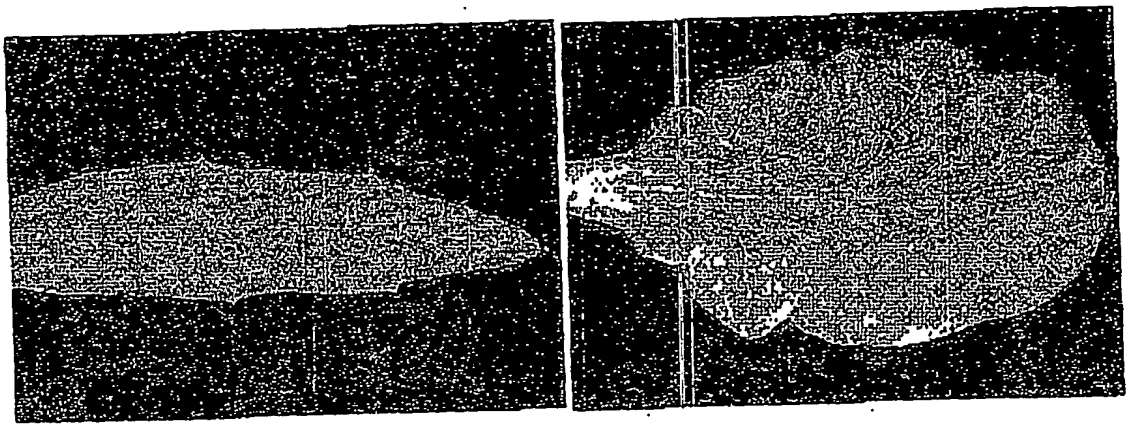
FIGURE 3



Wild type

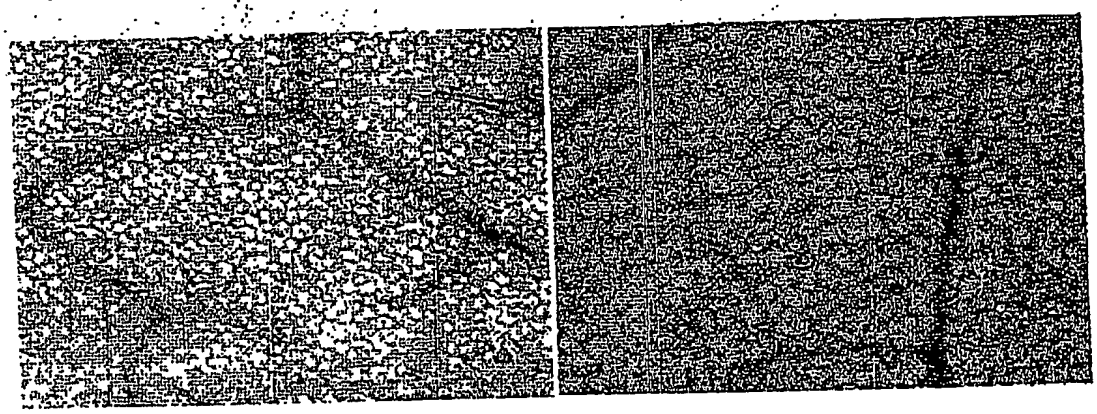
Transgenic

FIGURE 4



Wild type Transgenic

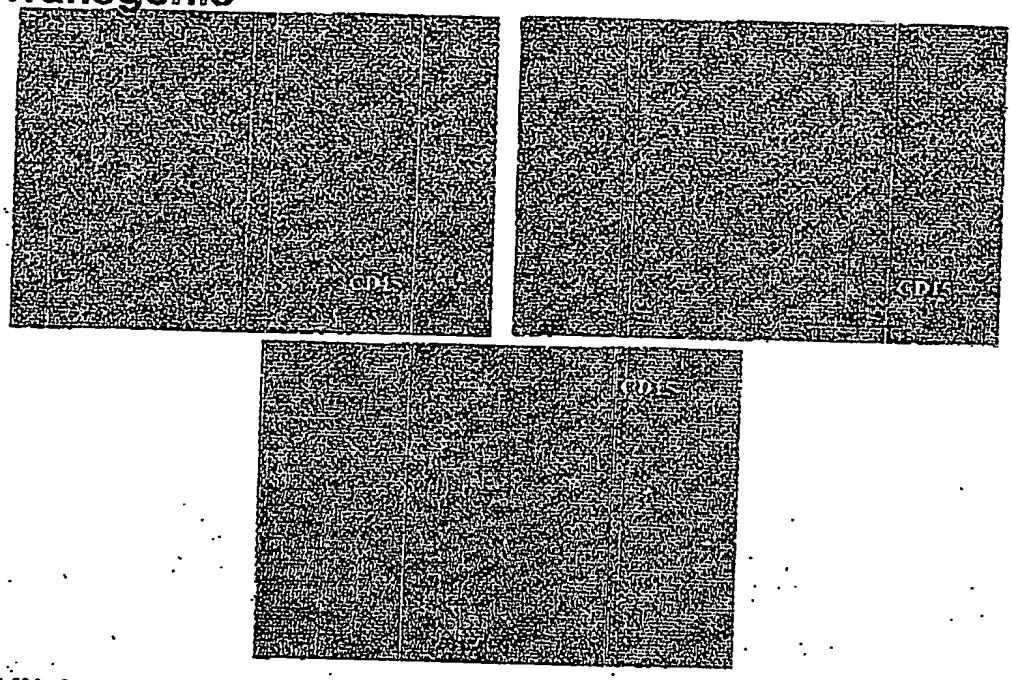
FIGURE 5



Wild type Transgenic

FIGURE 6

A Transgenic



B Wild type

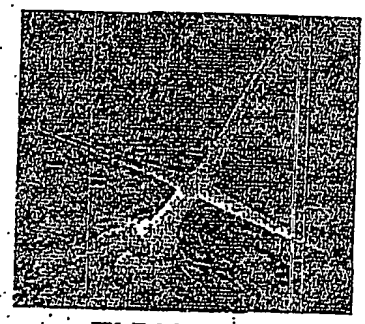
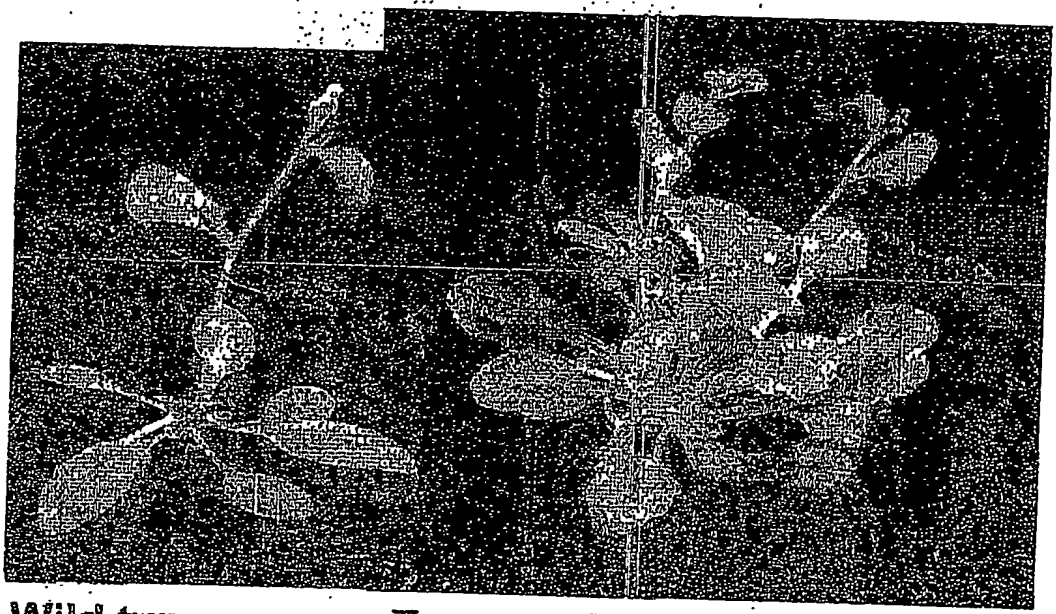


FIGURE 7

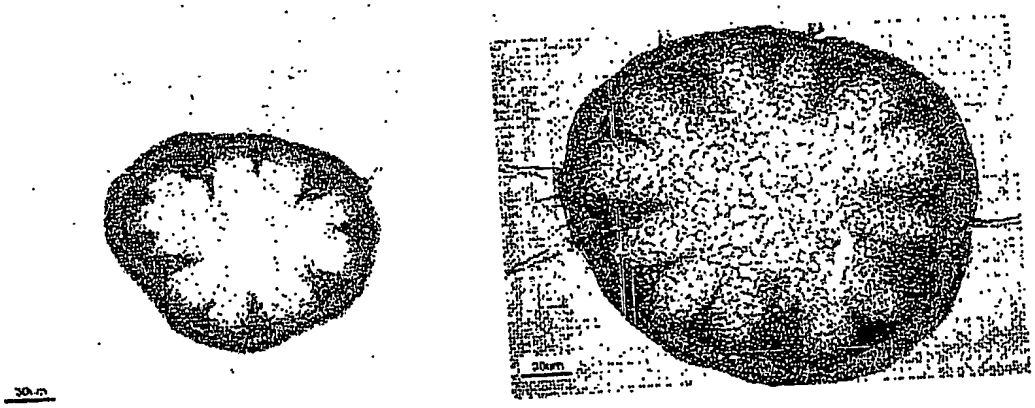


Wild type Transgenic

FIGURE 8

Wild type Transgenic

FIGURE 9



Wild type Transgenic

FIGURE 10

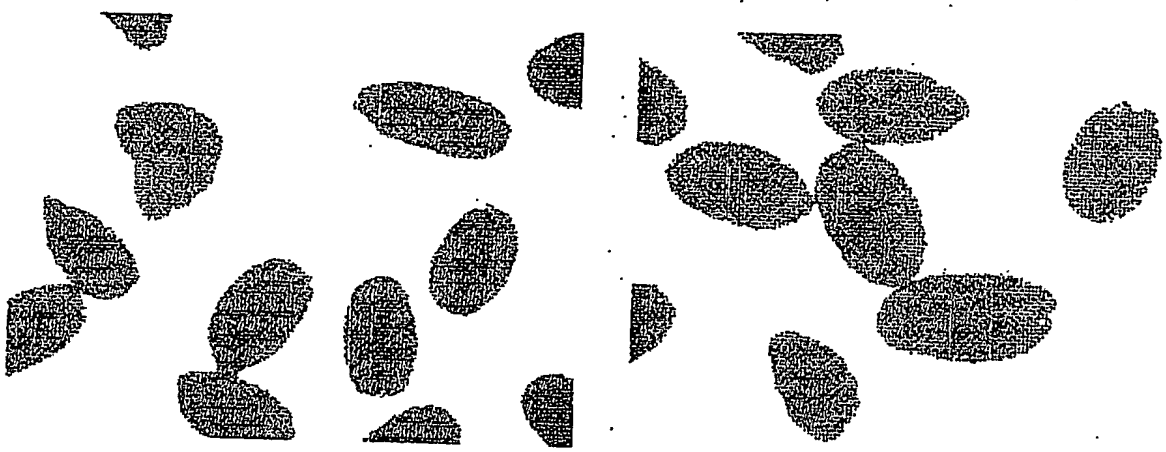


FIGURE 11

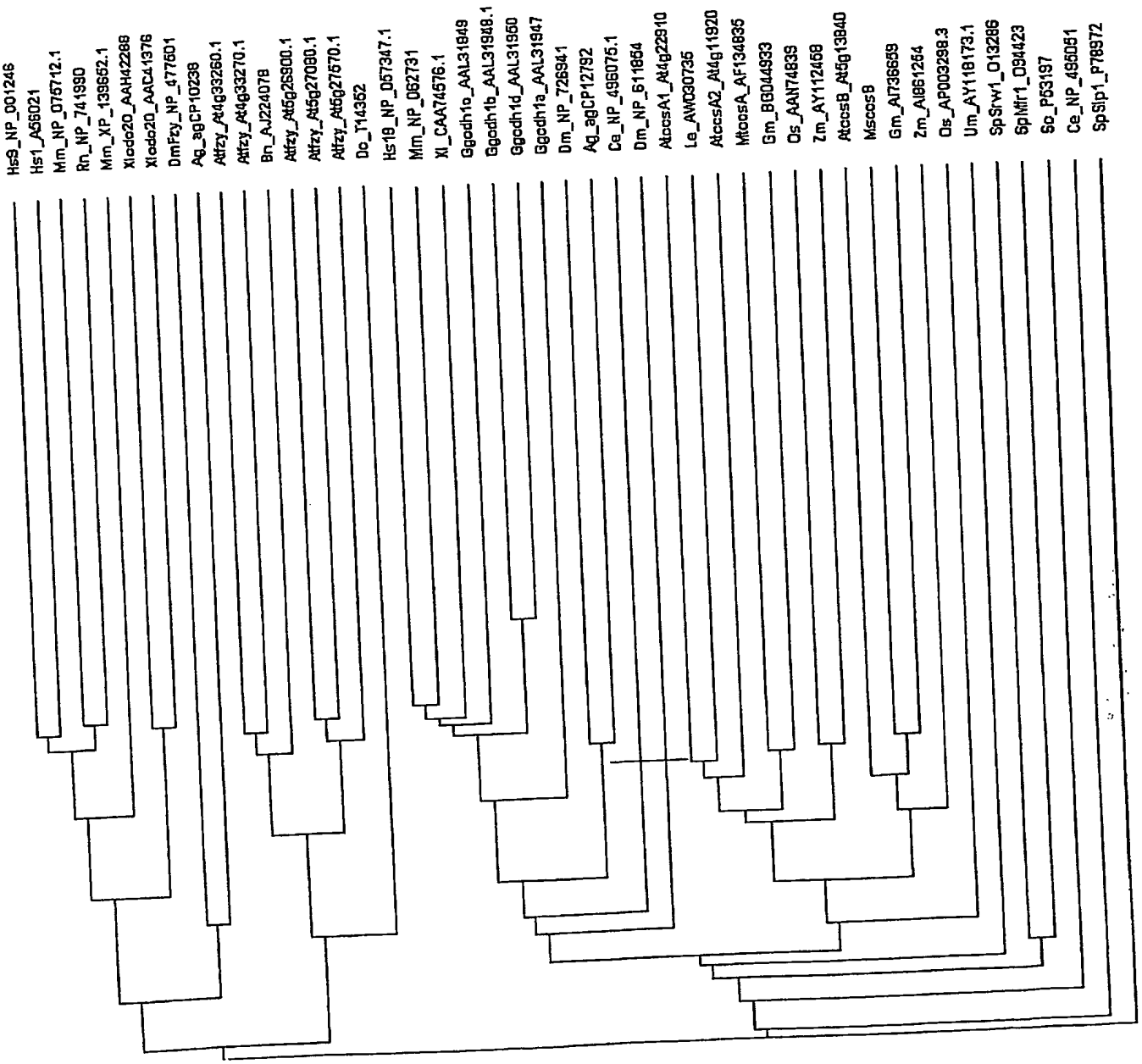


FIGURE 12

CCS52 motifs

Gene	Motif 1	Motif 2
AtCCS52A1 (At4g22910)	70 GSNFALFDL 78	88 EDGAGSYATLLRAAMFG 104
AtCCS52A2 (At4g11920)	61 GSNFALFDL 69	81 EDGAGSYASLLKTALFG 97
AtCCS52B (At5g13840)	54 SSRLHAFDL 62	73 EGGNEAYSRLLKSELFG 89
Rice CCS52A (AAN74839)	60 GSNLALFDL 68	94 TPASSPYCALLRAALFG 110
Consensus	XSXXXXFDL	XXXXXXYXXLLXXXXFG
SEQ ID NO	SEQ ID NO 7	SEQ ID NO 8

Motif 3	Motif 4
117 SSSRNIFRFKTETHRSL 133	207 SKVTKL 211
111 SPSGNIFRFKTETQRSL 127	198 SKVTKL 202
111 SPCTNMLRFKTDRSNS 129	203 SKVTKL 207
138 PATGNIFRFKAEVPRNA 153	229 SKVTKL 233
XXXXNXXRFKXXXRXXX	SKVTKL
SEQ ID NO 9	SEQ ID NO 10

Motif 5	Motif 6
289 DHVSKLAGHKS 300	329 HSTQPVLKYSEH 340
283 DHVSKLKGHKS 293	330 HSTQPVLRFCEH 341
288 DFVSKLVGHKS 298	335 HSQQPILKLTEH 346
313 DYISRLAGHKS 323	350 HSAHPVLKYTEH 361
DXXSXLXGHKS	HSXXPXLXXXEH
SEQ ID NO 11	SEQ ID NO 12

Motif 7	Motif 8	Motif 9
371 WNTTTNTHLSSIDT 384	403 LYLAVSPDGQTIVT 416	471 EIGSSFFGRTTIR 483
364 WNTTTNTHLNCVDT 377	426 LYLAVSPDGQTIVT 439	463 EIGALSFGRTTIR 476
369 WNTTNGNQLNSIDT 382	431 LYLATSPDGQTIVT 444	469 DTGLWSLGLTQIR 481
394 WNTTTNMHLNCVDT 107	456 LYLAISPDGQTIVT 469	495 SIGATSFVRSYIR 508
WNTTXXXXXXXXDT	LYLAXSPDGQTIVT	XXGXXXXXXXXIR
SEQ ID NO 13	SEQ ID NO 14	SEQ ID NO 15

FIGURE 13

SEQ ID NO 1: Arabidopsis thaliana cDNA encoding CCS52A1 At4g22910

ATGGAAGAAGAAGATCCTACAGCAAGCAATGTGATAACGAATTCGAATTCTTCATCTATGAG
AAACCTATCGCCGGCGATGAATACTCCGGTGGTTTCACTTGAGTCACGAATCAATCGATTAA
TCAATGCTAATCAATCTCAATCACCATCACCATCATCACTATCAAGGTCTATATACTCTGAT
AGATTTATCCCCAGTAGATCCGGATCCAATTTCGCTCTTTTCGATCTATCTCCTTCTCCTAG
TAAAGATGGTAAGGAAGATGGAGCTGGCTCTTACGCTACTCTGTTGCGTGCGGCGATGTTTG
GTCCTGAGACGCCGGAGAAGAGAGATATTACTGGGTTTTCTTCTTCCAGGAATATTTTTAGG
TTTAAGACGGAGACTCATCGGTCTTTGAATTCGTTTTCTCCTTTTGGTGTTGATGATGATTC
TCCTGGTGTTTCTCATAGTGGTCCTGTTAAAGCTCCCAGGAAAGTGCCGCGATCGCCGTATA
AGATTCTTGATCTCGTTGACTTTAGATCTTTGGTTTCGATAATGCATGAAACAATTTGTGAT
CTTTGTGATGTTTTGGTCTCTGAGGGTCTAGAATTTGAGTCTGAGGTATTGGATGCACCGGC
CTTGCAAGATGATTTTTATCTGAATCTTGTGGATTGGTCTGCACAAAATGTTCTAGCAGTGG
GACTAGGGAACTGTGTGTATTTATGGAATGCTTGTAGCAGCAAGGTTACTAAGTTATGTGAT
CTCGGAGCTGAGGATAGTGTTTGCTCAGTGGGTTGGGCGTTACGTGGAACTCATCTGGCTGT
TGGAACTAGTACCGGGAAAGTTCAGATATGGGATGCGTCACGCTGCAAGAGAACAAGAACAA
TGGAAGGTCATCGTCTAAGAGTTGGAGCCCTGGCATGGGGTTCATCGGTTCTGTCATCTGGT
AGCAGAGACAAGAGTATTCTTCAGAGAGACATAAGGTGTCAAGAAGATCATGTCAGTAAATT
GGCAGGTCATAAATCTGAGGTATGCGGACTCAAGTGGTCTTATGACAACAGAGAGCTAGCAT
CTGGTGGAAACGACAATAGGCTTTTTGTATGGAACCAACATTCAACACAACCGGTTTTGAAA
TATAGTGAACACACTGCAGCTGTTAAAGCCATTGCTTGGTCTCCTCATGTTCATGGGCTTCT
TGCTTCTGGTGGTGGTACTGCTGATAGATGCATACGTTTTTGGAATACAACCACGAATACTC
ATTTAAGTTCCATAGATACTTGCAGTCAGGTATGCAATCTAGCTTGGTCTAAGAACGTAAAC
GAGCTTGTTAGCACACACGGATACTCTCAGAACCAAATCATTGTCTGGAAATACCCAACCAT
GTCCAAAATTGCTACTCTAACCGGTCACACATACCGAGTCTTATACCTTGCGGTTTCACCCG
ATGGACAGACGATTGTAACAGGAGCAGGAGATGAAACCTTAAGGTTCTGGAATGTTTTCCCT
TCCCCAAAATCTCAGAACACGGATAGTGAAATCGGGTCGTCTTTCTTTGGTAGAACAACAAT
TCGGTGAGAAGTTACTTTCAAAACACACAGAAAAAGTCATAAATTCTTGATTTCTTCAGCAG
CAGCCAGCTTGAGTTGGTCGTCTCAACCAACTTTTTTCACACGGGAGCAGAGAGTCATTAAA
TTCTTTTACACACGGATGCAACAAGATCTAACCCTTTTGATTTAATCACGATCTTTGGGTTT
CCATCAAGATGCACAACATTTTCCCCCAAAATTTTCCAAAGTGTATATCTTTATTCAATTTT
TCTTCATATATCAAAATATAGTTTCTTTTGTATTTATTTACTTACGAACACAACATTTTATA
AAATAAGCCCATGATAATAATGCAATAATTCGTTACCATTCTCTT

SEQ ID NO 2: protein AtCCS52A1

MEEEDPTASNVITNSNSSSMRNLSPAMNTPVVSLESRINRLINANQSQSPSPSSLSRSIYSD
RFIPSRSGSNFALFDLSPSPSKDGKEDGAGSYATLLRAAMFGPETPEKRDITGFSSSRNIFR
FKTETHRSLNSFSPFGVDDDSPGVSHSGPVKAPRKVPRSPYKILDLVDFRSLVSIMHETICD
LCDVLVSEGLEFESEVLDAPALQDDFYLNLVDWSAQNVLAVGLGNCVYLWNACSSKVTKLCD
LGAEDSVCSVGWALRGTHLAVGTSTGKVQIWDASRCKRTRTMEGHRLRVGALAWGSSVLSSG
SRDKSILQRDIRCQEDHVSKLAGHKSEVCGLKWSYDNRELASGGNDNRLFVWNQHSTQPVLK
YSEHTAAVKAIAWSPHVHGLLASGGGTADRCIRFWNTTTNTHLSSIDTCSQVCNLAWSKNVN
ELVSTHGYSQNQIIVWKYPTMSKIATLTGHTYRVLYLAVSPDGQTIVTGAGDETLRFWNVFP
SPKSQNTDSEIG SSFFGRTTIR

FIGURE 14

SEQ ID NO 3: Oryza sativa cDNA encoding CCS52A AAN74839

ATGGAGAACTCCGCGTCCTCCAAGCCGCCCACCCCGGCGTCCACCCCGTCGTCGCGCCTCGC
CGCCGCGCCGTCCTCCCGCGTCTCCTCCGCGGCGCCGCACCCCTCCCCGTCCTCCTCCGCGC
CCACGCCGGCCTCGCGGACGGTCTACAGCGACCGCTTCATCCCCAGCCGCGCCGGATCCAAC
CTCGCGCTCTTCGACCTCGCCCCGTCGCCGTCCCACCACGACGCCGCCGCCGCCGCCGCCTC
CCCCGGCGCGCCGCCCCCCTCCGGATCTACCCCGGCCTCGTCGCCCTACTGCGCGCTCCTCC
GCGCCGCGCTCTTCGGCCCCACCACGCCCGACCGGGTGGCGTCGTCGGCGTCCGCGTGCTCC
TCCTCCTCCTCCGCCGGGGCGTCGCCCGTGGGCTCACCCGCCACCGGCAACATATTCAGGTT
CAAGGCGGAGGTGCCCCGGAATGCTAAGCGCGCCCTTTTCTCCGACGGGGACGACGAGGGCG
TGCTCTTCCCCGGGGGTGTTCACGACGAGGGGCACTGGCCCCAGGAAGATCCCTAGGTCACCT
TATAAGGTGCTGGATGCTCCCGCATTGCAGGATGACTTCTACCTGAACCTTGTGGATTGGTC
TTCGCATAATATCCTTGCAGTTGGATTGGGGAATTGTGTCTACTTATGGAATGCATGCAGCA
GCAAGGTCACCAAGCTATGTGATTTGGGGGTGGATGACAATGTCTGTTCAGTGGGTTGGGCA
CAGCGTGGCACTCACCTTGCTGTAGGGACAAACCAAGGCAAAGTTCAGGTATGGGATGCCAC
TCGTTGTAAGAGAATAAGAACCATGGAAAGCCATCGGATGCGAGTAGGTGCTCTTGCATGGA
ATTCATCATTGCTTTCGTCAGGCAGTCGTGACAAGAGCATCCTTCACCATGATATCCGTGCC
CAGGATGATTATATTAGTAGACTTGCTGGGCATAAATCGGAGGTCTGTGGGCTCAAGTGGTC
TTATGATAACCGTCAGCTTGCATCTGGTGGTAATGACAACAGACTTTATGTATGGAATCAAC
ACTCGGCGCACCCGGTACTGAAGTATACTGAGCATACAGCAGCTGTCAAAGCTATTGCGTGG
TCACCTCATCTTCATGGGCTGCTTGCATCTGGTGGAGGAACTGCAGATAGATGCATACGATT
TTGGAATACCACCACGAATATGCACTTAAATTGCGTCGACACAGGCAGTCAGGTCTGTAATC
TTGTATGGTCAAAGAATGTTAATGAGCTTGTTAGCACTCATGGATATTCTCAAAATCAGATA
ATTGTTTGGCGATACCCAACAATGTCAAAGCTCGCCACATTGACAGGCCATACATATAGGGT
ATTATATTTAGCCATCTCCCCAGATGGACAGACTATAGTAACTGGCGCTGGTGATGAAACGC
TTCGGTTTTGGAACGTGTTTCCATCTCCCAAGTCCCAGAGTTCTGACAGCCTAAGTAGCATC
GGGGCCACATCATTTGTTAGGAGCTACATCCGTCAACTTGGAGCTGGAGCTCTTATGTATAC
CATGCTAGGGCTTAACAACATTGGCCAACTCATGATGCTCATTGCATCCAAGTTGGAATATG
CTAAGGAAGCTGGAGAATTTCTGGTGCAATGTCCTATTCCAGCTGGTGTTGTTTCTTGCACT
GTTGCACTTCTCTGA

SEQ ID NO 4: Oryza sativa OsCCS52A protein

MENSASSKPPTPASTPSSRLAAAPSSRVSSAAPHPSPSSSAPTPASRTVYSDRFIPSRAGSN
LALFDLAPSPSHHDAAAAAASPGAPPPSGSTPASSPYCALLRAALFGPTTPDRVASSASACS
SSSSAGASPVGSPATGNIFRFKAEVPRNAKRALFSDGDDEGVLFPGVFTTRGTGPRKIPRSP
YKVLDAPALQDDFYLNLVDWSSHNILAVGLGNCVYLWNACSSKVTKLCDLGVDDNVCSVGWA
QRGTHLAVGTNQGKVQVWDATRCKRIRTMESHRMRVGALAWNSSLLSSGSRDKSILHHDIRA
QDDYISRLAGHKSEVCGLKWSYDNRQLASGGNDNRLYVWNQHSAHPVLKYTEHTAAVKAIAW
SPHLHGLLASGGGTADRCIRFWNTTTNMHLNCVDTGSQVCNLVWSKNVNELVSTHGYSQNQI
IVWRYPTMSKLATLTGHTYRVLYLAISPDGQTIVTGAGDETLRFWNVFPSPKSQSSDSLSSI
GATSFVRSYIRQLGAGALMYTMLGLNNIGQLMMLIASKLEYAKEAGEFLVQCPIPAGVVSCT
VALL

FIGURE 14 (continued)

SEQ ID NO 5: Oryza sativa genomic DNA AP003298 encoding CCS52-like protein

ATGCTAATGGGCCGGCCCGCATGGCAGAGAGAGTACAACGGCTACTCGGGTGGGGGGCCCAC
AGTCAGAGGGAGACAGCTCGTGCTAGAAAAAGTAGGCGACTTGCCCACTCCAACCAAAGTGA
CCGTTGCAACCTCATCTCCGCTCCTCTTCCTCCTCCTCGTCGTCGTTGTCGTCGTCGGCGGC
GCATCCAGCCTCGACGTGCCGGCGGCGCCGGCGCCGCCGCGCCTCAACGTGCCGCCGGCGAT
GGCGGGGGGGCTCCGCCTCGATCCCGCCGTCGCCTCCCCGGCCCGCCTCCTCCTCGACGTCC
CCAAGACGCCATCCCCTTCCAAGACCACGTACAGCGACCGCTTCATCCCCTGCCGCTCCTCC
TCCCGCCTCCACAACTTCGCCCTCCTCGACCGCGACCGCGCCTCCCCCTCCTCCACCACCGA
CGACGCCCCCTACTCCCGCCTCCTCCGCGCCGAGATCTTCGGCCCGGACTCCCCCTCCCCGG
CTCCCTCCTCCCCCAACACCAACCTCTTCCGCTTCAAGACCGACCACCCCTCGCCCAAATCG
CCCTTCGCCGCCTCCGCCGCCGCCACCGCCGGCCACTACGACTGCACCGCCGGCTCCGCTGA
ATCCTCCACGCCGCGCAAGCCGCCCAGGAAGGTCCCCAAGACCCCGCACAAGGTCCTGGACG
CGCCGTCGCTGCAGGACGACTTCTACCTCAATCTTGTCGACTGGTCGTCGCAGAACACGCTC
GCCGTCGGCCTCGGGAATTGCGTCTACCTCTGGTCGGCTTCCAATTGCAAGGTCACCAAGCT
CTGCGATTTGGGGCCCAGGGACAGCGTCTGCGCTGTGCACTGGACCCGAGAAGGCTCCTATC
TTGCCATCGGCACCAGCCTTGGCGATGTCCAGATTTGGGATAGCTCTCGCTGTAAACGGATT
AGGAACATGGGAGGACACCAAACACGGACTGGTGTATTAGCATGGAGCTCCCGAATCTTGTC
CTCCGGTAGCAGGGACAAGAACATATTGCAGCATGACATCCGTGTCCCAAGTGACTATATCA
GCAAGTTCTCAGGGCACAGATCAGAGAACCATGTATGTGCATCAAGTGACAGTTTTTTTGGT
CAGGTCTGTGGACTGAAATGGTCGCACGACGACCGTGAGCTTGCATCCGGTGGAAATGATAA
TCAGCTGCTAGTATGGAACCAACGTTCGCAGCAGCCGATATTGAGGCTGACAGAACACACAG
CTGCAGTTAAAGCAATAGCATGGTCACCACATCAGCAAGGCCTCCTGGCATCAGGTGGTGGA
ACCGCTGATAGGTGTATCAGGTTCTGGAACACGGTTAATGGAAACATGCTGAATTCAGTGGA
CACAGGCAGCCAGGCGACTTGTGAGCACTCATGGGTATTCCCAAAACCAAATCATGGTGTGG
AAGTACCCATCTATGTCAAAGGTTGCTACTCTAACTGGACACACGCTGCGAGTGCTTTACCT
TGCAATGTCACCACAATAGTAACAGGAGCCGGGGATGAAACCCTCAGATTTTGGAATATTTT
TCCTTCAATGAAGACACAGGTAGGCATCTATTGTTCA

SEQ ID NO 6: Oryza sativa protein BAB98864 corresponding to genomic DNA AP003298

MLMGRPAWQREYNGYSGGGPTVRGRQLVLEKVGDLPTPTKVTVATSSPLLFLLLVVVVVVGG
ASSLDVPAAPAPPRLNVPPAMAGGLRLDPAVASPARLLLDVPKTPSPSKTTYSDRFIPCRSS
SRLHNFALLDRDRASPSSTTDDAPYSRLLRAEIFGPDSPSPAPSSPNTNLFRFKTDHPSPKS
PFAASAAATAGHYDCTAGSAESSTPRKPPRKVPKTPHKVLDAPSLQDDFYLNLVDWSSQNTL
AVGLGNCVYLWSASNCKVTKLCDLGPRDSVCAVHWTREGSYLAIGTSLGDVQIWDSSRCKRI
RNMGGHQTRTGVLAWSSRILSSGSRDKNILQHDIRVPSDYISKFSGHRSENHVCASSDSFFG
QVCGLKWSHDDRELASGGNDNQLLVWNQRSQQPILRLTEHTAAVKAIAWSPHQQGLLASGGG
TADRCIRFWNTVNGNMLNSVDTGSQATCEHSWVFPKPNHGVEVPIYVKGCYSNWTHAASALP
CNVTTIVTGAGDETLRFWNIFPSMKTQVGIYC

SEQ ID NO 7: consensus motif 1 of CCS52 protein

XSXXXXFDL

FIGURE 14 (continued)

SEQ ID NO 8: consensus motif 2 of CCS52 protein

XXXXXXYXXLLXXXXFG

SEQ ID NO 9: consensus motif 3 of CCS52 protein

XXXXNXXRFKXXXRXXX

SEQ ID NO 10: consensus motif 4 of CCS52 protein

SKVTKL

SEQ ID NO 11: consensus motif 5 of CCS52 protein

DXXSXLXGHKS

SEQ ID NO 12: consensus motif 6 of CCS52 protein

HSXXPXLXXXEH

SEQ ID NO 13: consensus motif 7 of CCS52 protein

WNTTXXXXXXXXDT

SEQ ID NO 14: consensus motif 8 of CCS52 protein

LYLAXSPDGQTIVT

SEQ ID NO 15: consensus motif 9 of CCS52 protein

XXGXXXXXXXXIR

FIGURE 14 (continued)

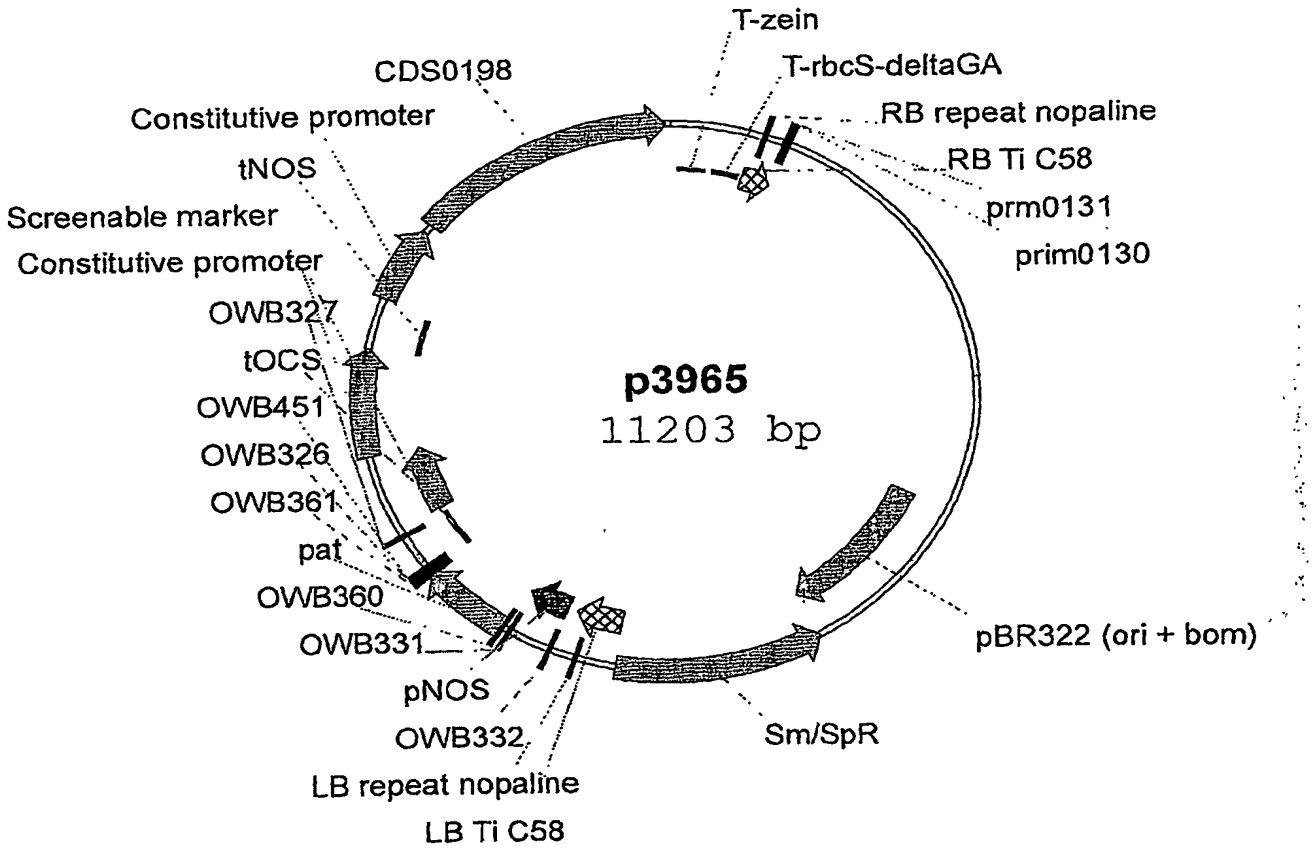

T-zein
T-rbcS-deltaGA
RB repeat nopaline
RB Ti C58
prm0131
prim0130
CDS0198
Constitutive promoter
tNOS
Screenable marker
Constitutive promoter
OWB327
tOCS
OWB451
OWB326
OWB361
pat
OWB360
OWB331
pNOS
OWB332
LB repeat nopaline
LB Ti C58
p3965
11203 bp
pBR322 (ori + bom)
Sm/SpR

FIGURE 15

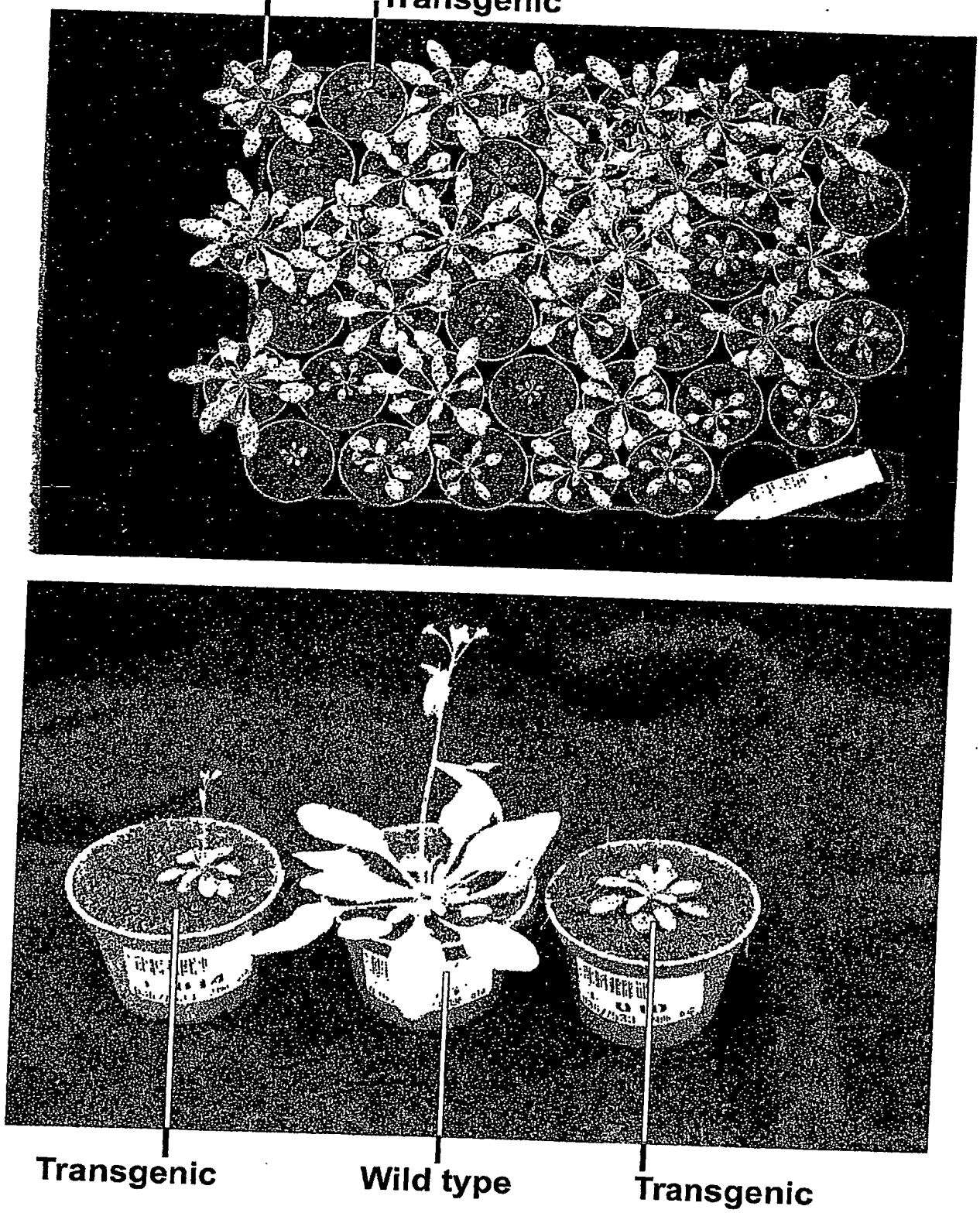


FIGURE 16

SEQUENCE LISTING

<110> CropDesign N.V.
Centre National de la Recherche Scientifique

<120> Plants having modified growth characteristics and a method for making the same

<130> 083-CCS52-PROV

<160> 15

<170> PatentIn version 3.1

<210> 1
<211> 1905
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> misc_feature
<223> cdNA encoding CCS52A1 At4g22910

<400> 1
atggaagaag aagatcctac agcaagcaat gtgataacga attcgaattc ttcatctatg 60

agaaacctat cgccggcgat gaatactccg gtggtttcac ttgagtcacg aatcaatcga 120

ttaatcaatg ctaatcaatc tcaatcacca tcaccatcat cactatcaag gtctatatac 180

tctgatagat ttatccccag tagatccgga tccaatttcg ctcttttcga tctatctcct 240

tctcctagta aagatggtaa ggaagatgga gctggctctt acgctactct gttgcgtgcg 300

gcgatgtttg gtcctgagac gccggagaag agagatatta ctgggttttc ttcttccagg 360

aatattttta ggtttaagac ggagactcat cggtctttga attcgttttc tccttttggt 420

gttgatgatg attctcctgg tgtttctcat agtggtcctg ttaaagctcc caggaaagtg 480

ccgcgatcgc cgtataagat tcttgatctc gttgacttta gatctttggt ttcgataatg 540

catgaaacaa tttgtgatct ttgtgatgtt ttggtctctg agggtctaga atttgagtct 600

gaggtattgg atgcaccggc cttgcaagat gatttttatc tgaatcttgt ggattggtct 660

gcacaaaatg ttctagcagt gggactaggg aactgtgtgt atttatggaa tgcttgtagc 720

agcaaggtta ctaagttatg tgatctcgga gctgaggata gtgtttgctc agtgggttgg
780

gcgttacgtg gaactcatct ggctgttgga actagtaccg ggaaagttca gatatgggat
840

gcgtcacgct gcaagagaac aagaacaatg gaaggtcatc gtctaagagt tggagccctg
900

gcatggggtt catcggttct gtcatctggt agcagagaca agagtattct tcagagagac
960

ataaggtgtc aagaagatca tgtcagtaaa ttggcaggtc ataaatctga ggtatgcgga
1020

ctcaagtggt cttatgacaa cagagagcta gcatctggtg gaaacgacaa taggcttttt
1080

gtatggaacc aacattcaac acaaccggtt ttgaaatata gtgaacacac tgcagctgtt
1140

aaagccattg cttggtctcc tcatgttcat gggcttcttg cttctggtgg tggtactgct
1200

gatagatgca tacgtttttg gaatacaacc acgaatactc atttaagttc catagatact
1260

tgcagtcagg tatgcaatct agcttggtct aagaacgtaa acgagcttgt tagcacacac
1320

ggatactctc agaaccaaat cattgtctgg aaatacccaa ccatgtccaa aattgctact
1380

ctaaccggtc acacataccg agtcttatac cttgcggttt cacccgatgg acagacgatt
1440

gtaacaggag caggagatga aaccttaagg ttctggaatg ttttcccttc cccaaaatct
1500

cagaacacgg atagtgaaat cgggtcgtct ttctttggta gaacaacaat tcggtgagaa
1560

gttactttca aaacacacag aaaaagtcat aaattcttga tttcttcagc agcagccagc
1620

ttgagttggt cgtctcaacc aacttttttc acacgggagc agagagtcat taaattcttt
1680

tacacacgga tgcaacaaga tctaaccctt ttgatttaat cacgatcttt gggtttccat
1740

caagatgcac aacattttcc cccaaaattt tccaaagtgt atatctttat tcaatttttc
1800

ttcatatatc aaaatatagt ttcttttgta tttatttact tacgaacaca acattttata
1860

aaataagccc atgataataa tgcaataatt cgttaccatt ctctt
1905

<210> 2
<211> 518
<212> PRT
<213> Arabidopsis thaliana

<220>
<221> MISC_FEATURE
<223> AtCCS52A1

<400> 2

Met Glu Glu Glu Asp Pro Thr Ala Ser Asn Val Ile Thr Asn Ser Asn
1 5 10 15

Ser Ser Ser Met Arg Asn Leu Ser Pro Ala Met Asn Thr Pro Val Val
20 25 30

Ser Leu Glu Ser Arg Ile Asn Arg Leu Ile Asn Ala Asn Gln Ser Gln
35 40 45

Ser Pro Ser Pro Ser Ser Leu Ser Arg Ser Ile Tyr Ser Asp Arg Phe
50 55 60

Ile Pro Ser Arg Ser Gly Ser Asn Phe Ala Leu Phe Asp Leu Ser Pro
65 70 75 80

Ser Pro Ser Lys Asp Gly Lys Glu Asp Gly Ala Gly Ser Tyr Ala Thr
85 90 95

Leu Leu Arg Ala Ala Met Phe Gly Pro Glu Thr Pro Glu Lys Arg Asp
100 105 110

Ile Thr Gly Phe Ser Ser Ser Arg Asn Ile Phe Arg Phe Lys Thr Glu
115 120 125

Thr His Arg Ser Leu Asn Ser Phe Ser Pro Phe Gly Val Asp Asp Asp
130 135 140

Ser Pro Gly Val Ser His Ser Gly Pro Val Lys Ala Pro Arg Lys Val
145 150 155 160

Pro Arg Ser Pro Tyr Lys Ile Leu Asp Leu Val Asp Phe Arg Ser Leu
165 170 175

Val Ser Ile Met His Glu Thr Ile Cys Asp Leu Cys Asp Val Leu Val
180 185 190

Ser Glu Gly Leu Glu Phe Glu Ser Glu Val Leu Asp Ala Pro Ala Leu

195 200 205

Gln Asp Asp Phe Tyr Leu Asn Leu Val Asp Trp Ser Ala Gln Asn Val
210 215 220

Leu Ala Val Gly Leu Gly Asn Cys Val Tyr Leu Trp Asn Ala Cys Ser
225 230 235 240

Ser Lys Val Thr Lys Leu Cys Asp Leu Gly Ala Glu Asp Ser Val Cys
245 250 255

Ser Val Gly Trp Ala Leu Arg Gly Thr His Leu Ala Val Gly Thr Ser
260 265 270

Thr Gly Lys Val Gln Ile Trp Asp Ala Ser Arg Cys Lys Arg Thr Arg
275 280 285

Thr Met Glu Gly His Arg Leu Arg Val Gly Ala Leu Ala Trp Gly Ser
290 295 300

Ser Val Leu Ser Ser Gly Ser Arg Asp Lys Ser Ile Leu Gln Arg Asp
305 310 315 320

Ile Arg Cys Gln Glu Asp His Val Ser Lys Leu Ala Gly His Lys Ser
325 330 335

Glu Val Cys Gly Leu Lys Trp Ser Tyr Asp Asn Arg Glu Leu Ala Ser
340 345 350

Gly Gly Asn Asp Asn Arg Leu Phe Val Trp Asn Gln His Ser Thr Gln
355 360 365

Pro Val Leu Lys Tyr Ser Glu His Thr Ala Ala Val Lys Ala Ile Ala
370 375 380

Trp Ser Pro His Val His Gly Leu Leu Ala Ser Gly Gly Gly Thr Ala
385 390 395 400

Asp Arg Cys Ile Arg Phe Trp Asn Thr Thr Thr Asn Thr His Leu Ser
405 410 415

Ser Ile Asp Thr Cys Ser Gln Val Cys Asn Leu Ala Trp Ser Lys Asn
420 425 430

Val Asn Glu Leu Val Ser Thr His Gly Tyr Ser Gln Asn Gln Ile Ile
435 440 445

Val Trp Lys Tyr Pro Thr Met Ser Lys Ile Ala Thr Leu Thr Gly His
450 455 460

Thr Tyr Arg Val Leu Tyr Leu Ala Val Ser Pro Asp Gly Gln Thr Ile
465 470 475 480

Val Thr Gly Ala Gly Asp Glu Thr Leu Arg Phe Trp Asn Val Phe Pro
485 490 495

Ser Pro Lys Ser Gln Asn Thr Asp Ser Glu Ile Gly Ser Ser Phe Phe
500 505 510

Gly Arg Thr Thr Ile Arg
515

<210> 3
<211> 1689
<212> DNA
<213> Oryza sativa

<220>
<221> misc_feature
<223> cDNA encoding CCS52A AAN74839

<400> 3
atggagaact ccgcgtcctc caagccgccc accccggcgt ccaccccgtc gtcgcgcctc
60

gccgccgcgc cgtcctcccg cgtctcctcc gcggcgccgc acccctcccc gtcctcctcc
120

gcgcccacgc cggcctcgcg gacggtctac agcgaccgct tcatccccag ccgcgccgga
180

tccaacctcg cgctcttcga cctcgccccg tcgccgtccc accacgacgc cgccgccgcc
240

gccgcctccc ccggcgcgcc gccccccctcc ggatctaccc cggcctcgtc gccctactgc
300

gcgctcctcc gcgccgcgct cttcggcccc accacgcccg accgggtggc gtcgtcggcg
360

tccgcgtgct cctcctcctc ctccgccggg gcgtcgcccg tgggctcacc cgccaccggc
420

aacatattca ggttcaaggc ggaggtgccc cggaatgcta agcgcgccct tttctccgac
480

ggggacgacg agggcgtgct cttccccggg gtgttcacga cgaggggcac tggccccagg
540

aagatcccta ggtcaccttа taaggtgctg gatgctcccg cattgcagga tgacttctac
600

ctgaaccttg tggattggtc ttcgcataat atccttgcag ttggattggg gaattgtgtc
660

tacttatgga atgcatgcag cagcaaggtc accaagctat gtgatttggg ggtggatgac
720

aatgtctgtt cagtgggttg ggcacagcgt ggcactcacc ttgctgtagg gacaaaccaa
780

ggcaaagttc aggtatggga tgccactcgt tgtaagagaa taagaaccat ggaaagccat
840

cggatgcgag taggtgctct tgcatggaat tcatcattgc tttcgtcagg cagtcgtgac
900

aagagcatcc ttcaccatga tatccgtgcc caggatgatt atattagtag acttgctggg
960

cataaatcgg aggtctgtgg gctcaagtgg tcttatgata accgtcagct tgcatctggt
1020

ggtaatgaca acagacttta tgtatggaat caacactcgg cgcacccggt actgaagtat
1080

actgagcata cagcagctgt caaagctatt gcgtggtcac ctcatcttca tgggctgctt
1140

gcatctggtg gaggaactgc agatagatgc atacgatttt ggaataccac cacgaatatg
1200

cacttaaatt gcgtcgacac aggcagtcag gtctgtaatc ttgtatggtc aaagaatgtt
1260

aatgagcttg ttagcactca tggatattct caaaatcaga taattgtttg gcgataccca
1320

acaatgtcaa agctcgccac attgacaggc catacatata gggtattata tttagccatc
1380

tccccagatg gacagactat agtaactggc gctggtgatg aaacgcttcg gttttggaac
1440

gtgtttccat ctcccaagtc ccagagttct gacagcctaa gtagcatcgg ggccacatca
1500

tttgttagga gctacatccg tcaacttgga gctggagctc ttatgtatac catgctaggg
1560

cttaacaaca ttggccaact catgatgctc attgcatcca agttggaata tgctaaggaa
1620

gctggagaat ttctggtgca atgtcctatt ccagctggtg ttgtttcttg cactgttgca
1680

cttctctga
1689

<210> 4
<211> 562
<212> PRT
<213> Oryza sativa

<220>
<221> MISC_FEATURE
<223> OsCCS52A

<400> 4

Met Glu Asn Ser Ala Ser Ser Lys Pro Pro Thr Pro Ala Ser Thr Pro
1 5 10 15

Ser Ser Arg Leu Ala Ala Ala Pro Ser Ser Arg Val Ser Ser Ala Ala
20 25 30

Pro His Pro Ser Pro Ser Ser Ser Ala Pro Thr Pro Ala Ser Arg Thr
35 40 45

Val Tyr Ser Asp Arg Phe Ile Pro Ser Arg Ala Gly Ser Asn Leu Ala
50 55 60

Leu Phe Asp Leu Ala Pro Ser Pro Ser His His Asp Ala Ala Ala Ala
65 70 75 80

Ala Ala Ser Pro Gly Ala Pro Pro Pro Ser Gly Ser Thr Pro Ala Ser
85 90 95

Ser Pro Tyr Cys Ala Leu Leu Arg Ala Ala Leu Phe Gly Pro Thr Thr
100 105 110

Pro Asp Arg Val Ala Ser Ser Ala Ser Ala Cys Ser Ser Ser Ser Ser
115 120 125

Ala Gly Ala Ser Pro Val Gly Ser Pro Ala Thr Gly Asn Ile Phe Arg
130 135 140

Phe Lys Ala Glu Val Pro Arg Asn Ala Lys Arg Ala Leu Phe Ser Asp
145 150 155 160

Gly Asp Asp Glu Gly Val Leu Phe Pro Gly Val Phe Thr Thr Arg Gly
165 170 175

Thr Gly Pro Arg Lys Ile Pro Arg Ser Pro Tyr Lys Val Leu Asp Ala
180 185 190

Pro Ala Leu Gln Asp Asp Phe Tyr Leu Asn Leu Val Asp Trp Ser Ser

195 200 205

His Asn Ile Leu Ala Val Gly Leu Gly Asn Cys Val Tyr Leu Trp Asn
210 215 220

Ala Cys Ser Ser Lys Val Thr Lys Leu Cys Asp Leu Gly Val Asp Asp
225 230 235 240

Asn Val Cys Ser Val Gly Trp Ala Gln Arg Gly Thr His Leu Ala Val
245 250 255

Gly Thr Asn Gln Gly Lys Val Gln Val Trp Asp Ala Thr Arg Cys Lys
260 265 270

Arg Ile Arg Thr Met Glu Ser His Arg Met Arg Val Gly Ala Leu Ala
275 280 285

Trp Asn Ser Ser Leu Leu Ser Ser Gly Ser Arg Asp Lys Ser Ile Leu
290 295 300

His His Asp Ile Arg Ala Gln Asp Asp Tyr Ile Ser Arg Leu Ala Gly
305 310 315 320

His Lys Ser Glu Val Cys Gly Leu Lys Trp Ser Tyr Asp Asn Arg Gln
325 330 335

Leu Ala Ser Gly Gly Asn Asp Asn Arg Leu Tyr Val Trp Asn Gln His
340 345 350

Ser Ala His Pro Val Leu Lys Tyr Thr Glu His Thr Ala Ala Val Lys
355 360 365

Ala Ile Ala Trp Ser Pro His Leu His Gly Leu Leu Ala Ser Gly Gly
370 375 380

Gly Thr Ala Asp Arg Cys Ile Arg Phe Trp Asn Thr Thr Thr Asn Met
385 390 395 400

His Leu Asn Cys Val Asp Thr Gly Ser Gln Val Cys Asn Leu Val Trp
405 410 415

Ser Lys Asn Val Asn Glu Leu Val Ser Thr His Gly Tyr Ser Gln Asn
420 425 430

Gln Ile Ile Val Trp Arg Tyr Pro Thr Met Ser Lys Leu Ala Thr Leu
435 440 445

```
Thr Gly His Thr Tyr Arg Val Leu Tyr Leu Ala Ile Ser Pro Asp Gly
    450                 455                 460

Gln Thr Ile Val Thr Gly Ala Gly Asp Glu Thr Leu Arg Phe Trp Asn
465                 470                 475                 480

Val Phe Pro Ser Pro Lys Ser Gln Ser Ser Asp Ser Leu Ser Ser Ile
                485                 490                 495

Gly Ala Thr Ser Phe Val Arg Ser Tyr Ile Arg Gln Leu Gly Ala Gly
            500                 505                 510

Ala Leu Met Tyr Thr Met Leu Gly Leu Asn Asn Ile Gly Gln Leu Met
        515                 520                 525

Met Leu Ile Ala Ser Lys Leu Glu Tyr Ala Lys Glu Ala Gly Glu Phe
    530                 535                 540

Leu Val Gln Cys Pro Ile Pro Ala Gly Val Val Ser Cys Thr Val Ala
545                 550                 555                 560

Leu Leu
```

<210> 5
<211> 1587
<212> DNA
<213> Oryza sativa

<220>
<221> misc_feature
<223> genomic DNA AP003298 encoding CCS52-like protein

<400> 5
atgctaatgg gccggcccgc atggcagaga gagtacaacg gctactcggg tgggggccc
60

acagtcagag ggagacagct cgtgctagaa aaagtaggcg acttgcccac tccaaccaaa
120

gtgaccgttg caacctcatc tccgctcctc ttcctcctcc tcgtcgtcgt tgtcgtcgtc
180

ggcggcgcat ccagcctcga cgtgccggcg gcgccggcgc cgccgcgcct caacgtgccg
240

ccggcgatgg cgggggggct ccgcctcgat cccgccgtcg cctccccggc ccgcctcctc
300

ctcgacgtcc ccaagacgcc atccccttcc aagaccacgt acagcgaccg cttcatcccc
360

tgccgctcct cctcccgcct ccacaacttc gccctcctcg accgcgaccg cgcctccccc
420

tcctccacca ccgacgacgc cccctactcc cgcctcctcc gcgccgagat cttcggcccg
480

gactcccccc ccccggctcc ctcctccccc aacaccaacc tcttccgctt caagaccgac
540

cacccctcgc ccaaatcgcc cttcgccgcc tccgccgccg ccaccgccgg ccactacgac
600

tgcaccgccg gctccgctga atcctccacg ccgcgcaagc cgcccaggaa ggtccccaag
660

accccgcaca aggtcctgga cgcgccgtcg ctgcaggacg acttctacct caatcttgtc
720

gactggtcgt cgcagaacac gctcgccgtc ggcctcggga attgcgtcta cctctggtcg
780

gcttccaatt gcaaggtcac caagctctgc gatttggggc ccagggacag cgtctgcgct
840

gtgcactgga cccgagaagg ctcctatctt gccatcggca ccagccttgg cgatgtccag
900

atttgggata gctctcgctg taaacggatt aggaacatgg gaggacacca aacacggact
960

ggtgtattag catggagctc ccgaatcttg tcctccggta gcagggacaa gaacatattg
1020

cagcatgaca tccgtgtccc aagtgactat atcagcaagt tctcagggca cagatcagag
1080

aaccatgtat gtgcatcaag tgacagtttt tttggtcagg tctgtggact gaaatggtcg
1140

cacgacgacc gtgagcttgc atccggtgga aatgataatc agctgctagt atggaaccaa
1200

cgttcgcagc agccgatatt gaggctgaca gaacacacag ctgcagttaa agcaatagca
1260

tggtcaccac atcagcaagg cctcctggca tcaggtggtg gaaccgctga taggtgtatc
1320

aggttctgga acacggttaa tggaaacatg ctgaattcag tggacacagg cagccaggcg
1380

acttgtgagc actcatgggt attcccaaaa ccaaatcatg gtgtggaagt acccatctat
1440

gtcaaaggtt gctactctaa ctggacacac gctgcgagtg ctttaccttg caatgtcacc
1500

acaatagtaa caggagccgg ggatgaaacc ctcagatttt ggaatatttt tccttcaatg
1560

aagacacagg taggcatcta ttgttga
1587

<210> 6
<211> 528
<212> PRT
<213> Oryza sativa

<220>
<221> MISC_FEATURE
<223> BAB98864 corresponding to genomic DNA AP03298

<400> 6

Met Leu Met Gly Arg Pro Ala Trp Gln Arg Glu Tyr Asn Gly Tyr Ser
1 5 10 15

Gly Gly Gly Pro Thr Val Arg Gly Arg Gln Leu Val Leu Glu Lys Val
20 25 30

Gly Asp Leu Pro Thr Pro Thr Lys Val Thr Val Ala Thr Ser Ser Pro
35 40 45

Leu Leu Phe Leu Leu Leu Val Val Val Val Val Val Gly Gly Ala Ser
50 55 60

Ser Leu Asp Val Pro Ala Ala Pro Ala Pro Pro Arg Leu Asn Val Pro
65 70 75 80

Pro Ala Met Ala Gly Gly Leu Arg Leu Asp Pro Ala Val Ala Ser Pro
85 90 95

Ala Arg Leu Leu Leu Asp Val Pro Lys Thr Pro Ser Pro Ser Lys Thr
100 105 110

Thr Tyr Ser Asp Arg Phe Ile Pro Cys Arg Ser Ser Ser Arg Leu His
115 120 125

Asn Phe Ala Leu Leu Asp Arg Asp Arg Ala Ser Pro Ser Ser Thr Thr
130 135 140

Asp Asp Ala Pro Tyr Ser Arg Leu Leu Arg Ala Glu Ile Phe Gly Pro
145 150 155 160

Asp Ser Pro Ser Pro Ala Pro Ser Ser Pro Asn Thr Asn Leu Phe Arg
165 170 175

Phe Lys Thr Asp His Pro Ser Pro Lys Ser Pro Phe Ala Ala Ser Ala
180 185 190

Ala Ala Thr Ala Gly His Tyr Asp Cys Thr Ala Gly Ser Ala Glu Ser
195 200 205

Ser Thr Pro Arg Lys Pro Pro Arg Lys Val Pro Lys Thr Pro His Lys
210 215 220

Val Leu Asp Ala Pro Ser Leu Gln Asp Asp Phe Tyr Leu Asn Leu Val
225 230 235 240

Asp Trp Ser Ser Gln Asn Thr Leu Ala Val Gly Leu Gly Asn Cys Val
245 250 255

Tyr Leu Trp Ser Ala Ser Asn Cys Lys Val Thr Lys Leu Cys Asp Leu
260 265 270

Gly Pro Arg Asp Ser Val Cys Ala Val His Trp Thr Arg Glu Gly Ser
275 280 285

Tyr Leu Ala Ile Gly Thr Ser Leu Gly Asp Val Gln Ile Trp Asp Ser
290 295 300

Ser Arg Cys Lys Arg Ile Arg Asn Met Gly Gly His Gln Thr Arg Thr
305 310 315 320

Gly Val Leu Ala Trp Ser Ser Arg Ile Leu Ser Ser Gly Ser Arg Asp
325 330 335

Lys Asn Ile Leu Gln His Asp Ile Arg Val Pro Ser Asp Tyr Ile Ser
340 345 350

Lys Phe Ser Gly His Arg Ser Glu Asn His Val Cys Ala Ser Ser Asp
355 360 365

Ser Phe Phe Gly Gln Val Cys Gly Leu Lys Trp Ser His Asp Asp Arg
370 375 380

Glu Leu Ala Ser Gly Gly Asn Asp Asn Gln Leu Leu Val Trp Asn Gln
385 390 395 400

Arg Ser Gln Gln Pro Ile Leu Arg Leu Thr Glu His Thr Ala Ala Val
405 410 415

Lys Ala Ile Ala Trp Ser Pro His Gln Gln Gly Leu Leu Ala Ser Gly
420 425 430

Gly Gly Thr Ala Asp Arg Cys Ile Arg Phe Trp Asn Thr Val Asn Gly
435 440 445

Asn Met Leu Asn Ser Val Asp Thr Gly Ser Gln Ala Thr Cys Glu His
450 455 460

Ser Trp Val Phe Pro Lys Pro Asn His Gly Val Glu Val Pro Ile Tyr
465 470 475 480

Val Lys Gly Cys Tyr Ser Asn Trp Thr His Ala Ala Ser Ala Leu Pro
485 490 495

Cys Asn Val Thr Thr Ile Val Thr Gly Ala Gly Asp Glu Thr Leu Arg
500 505 510

Phe Trp Asn Ile Phe Pro Ser Met Lys Thr Gln Val Gly Ile Tyr Cys
515 520 525

<210> 7
<211> 9
<212> PRT
<213> Artificial sequence

<220>
<223> consensus motif 1 of CCS52 protein

<220>
<221> MISC_FEATURE
<222> (1)..(1)
<223> unknown amino acid

<220>
<221> MISC_FEATURE
<222> (3)..(6)
<223> unknown amino acid

<400> 7

Xaa Ser Xaa Xaa Xaa Xaa Phe Asp Leu
1 5

<210> 8
<211> 17
<212> PRT
<213> Artificial sequence

<220>

<223> consensus motif 2 of CCS52 protein

<220>
<221> MISC_FEATURE
<222> (1)..(6)
<223> unknown amino acid

<220>
<221> MISC_FEATURE
<222> (8)..(9)
<223> unknown amino acid

<220>
<221> MISC_FEATURE
<222> (12)..(15)
<223> unknown amino acid

<400> 8

Xaa Xaa Xaa Xaa Xaa Xaa Tyr Xaa Xaa Leu Leu Xaa Xaa Xaa Xaa Phe
1 5 10 15

Gly

<210> 9
<211> 17
<212> PRT
<213> Artificial sequence

<220>
<223> consensus motif 3 of CCS52 protein

<220>
<221> MISC_FEATURE
<222> (1)..(4)
<223> unknown amino acid

<220>
<221> MISC_FEATURE
<222> (6)..(7)
<223> unknown amino acid

<220>
<221> MISC_FEATURE
<222> (11)..(13)
<223> unknown amino acid

<220>
<221> MISC_FEATURE
<222> (15)..(17)
<223> unknown amino acid

<400> 9

Xaa Xaa Xaa Xaa Asn Xaa Xaa Arg Phe Lys Xaa Xaa Xaa Arg Xaa Xaa
1 5 10 15

Xaa

<210> 10
<211> 6
<212> PRT
<213> Artificial sequence

<220>
<223> consensus motif 4 of CCS52 protein

<400> 10

Ser Lys Val Thr Lys Leu
1 5

<210> 11
<211> 11
<212> PRT
<213> Artificial sequence

<220>
<223> consensus motif 5 of CCS52 protein

<220>
<221> MISC_FEATURE
<222> (2)..(3)
<223> unknown amino acid

<220>
<221> MISC_FEATURE
<222> (5)..(5)
<223> unknown amino acid

<220>
<221> MISC_FEATURE
<222> (7)..(7)
<223> unknown amino acid

<400> 11

Asp Xaa Xaa Ser Xaa Leu Xaa Gly His Lys Ser
1 5 10

<210> 12
<211> 12
<212> PRT
<213> Artificial sequence

<220>
<223> consensus motif 6 of CCS52 protein

<220>
<221> MISC_FEATURE
<222> (3)..(4)
<223> unknown amino acid

<220>
<221> MISC_FEATURE
<222> (6)..(6)
<223> unknown amino acid

<220>
<221> MISC_FEATURE
<222> (8)..(10)
<223> unknown amino acid

<400> 12

His Ser Xaa Xaa Pro Xaa Leu Xaa Xaa Xaa Glu His
1 5 10

<210> 13
<211> 14
<212> PRT
<213> Artificial sequence

<220>
<223> consensus motif 7 of CCS52 protein

<220>
<221> MISC_FEATURE
<222> (5)..(12)
<223> consensus motif 7 of CCS52 protein

<400> 13

Trp Asn Thr Thr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Thr
1 5 10

<210> 14
<211> 14
<212> PRT
<213> Artificial sequence

<220>
<223> consensus motif 8 of CCS52 protein

<220>
<221> MISC_FEATURE
<222> (5)..(5)
<223> unknown amino acid

<400> 14

Leu Tyr Leu Ala Xaa Ser Pro Asp Gly Gln Thr Ile Val Thr
1 5 10

<210> 15
<211> 13
<212> PRT
<213> Artificial sequence

<220>
<223> consensus motif 9 of CCS52 protein

<220>
<221> MISC_FEATURE
<222> (1)..(2)
<223> unknown amino acid

<220>
<221> MISC_FEATURE
<222> (4)..(11)
<223> unknown amino acid

<400> 15

Xaa Xaa Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ile Arg
1 5 10